APPENDICES

APPENDIX A

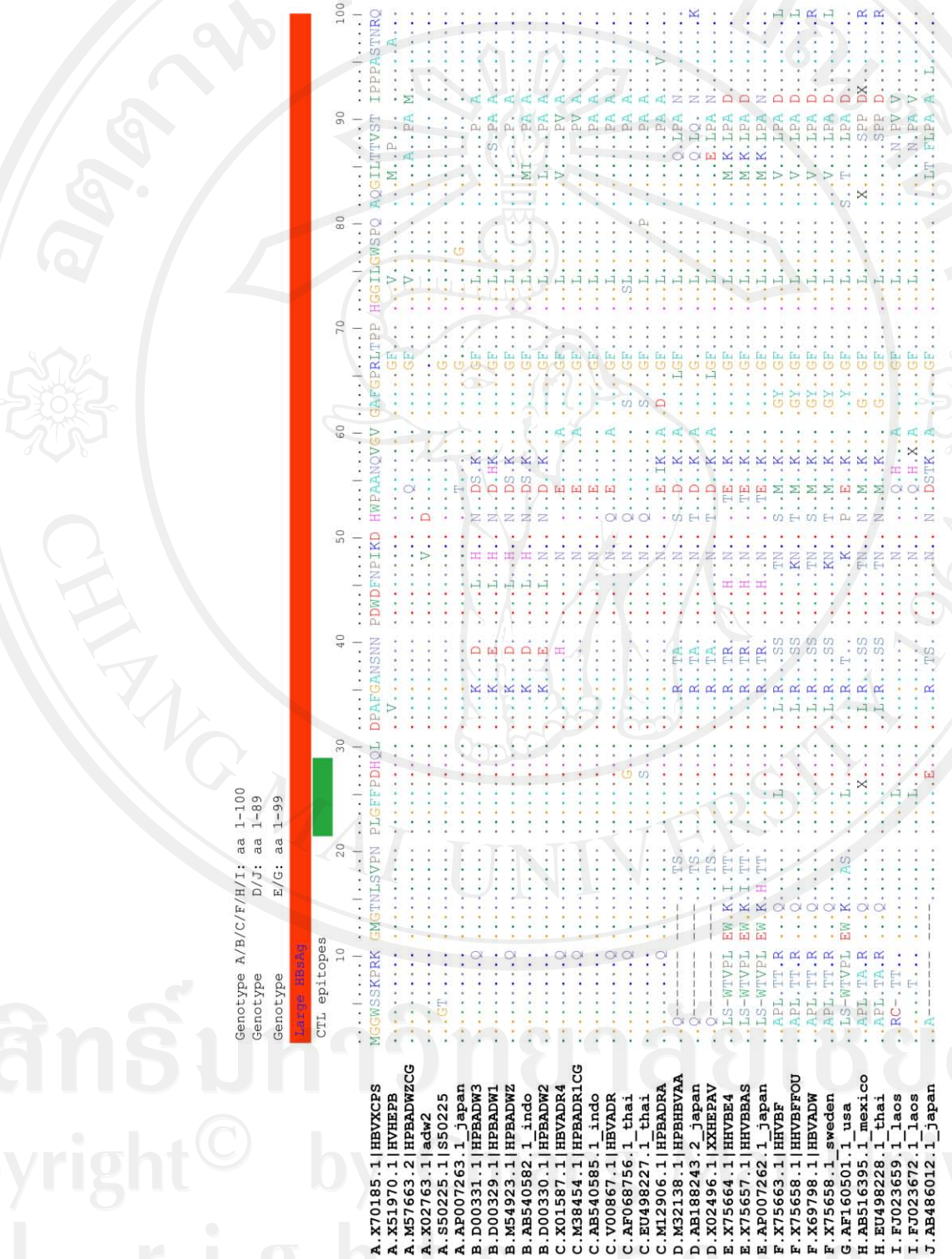
Table of standard amino acid abbreviations and properties

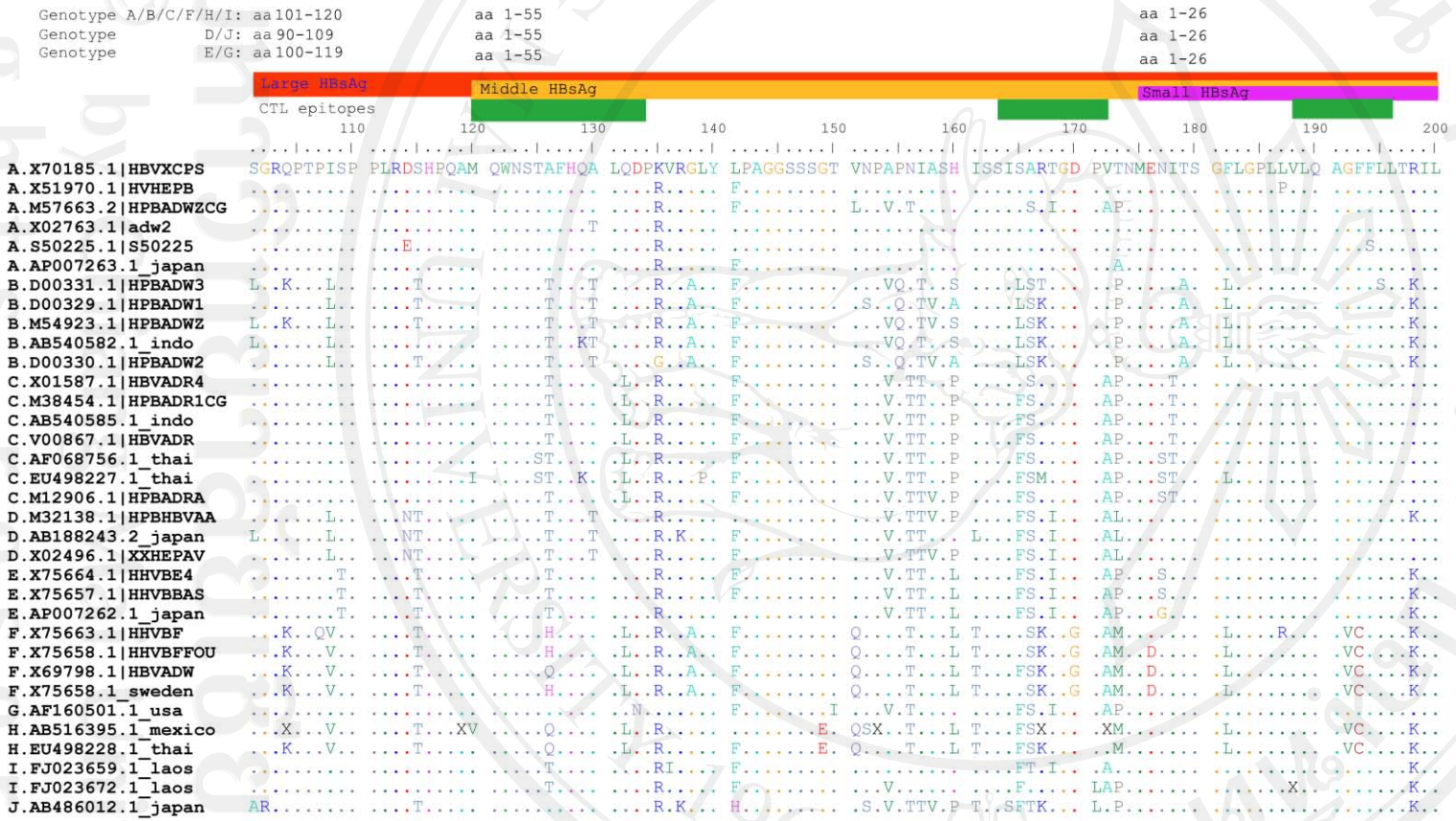
Amino Acid	3-Letter	1-Letter	Side-chain polarity	Side-chain charge (pH 7.4)	Hydropathy index
Alanine	Ala	A	nonpolar	neutral	1.8
Arginine	Arg	R	polar	positive	−4.5
Asparagine	Asn	N	polar	neutral	−3.5
Aspartic acid	Asp	D	polar	negative	−3.5
Cysteine	Cys	C	polar	neutral	2.5
Glutamic acid	Glu	E	polar	negative	−3.5
Glutamine	Gln	Q	polar	neutral	−3.5
Glycine	Gly	G	nonpolar	neutral	−0.4
Histidine	His	H	polar	positive (10%), neutral (90%)	−3.2
Isoleucine	Ile	I	nonpolar	neutral	4.5
Leucine	Leu	L	nonpolar	neutral	3.8
Lysine	Lys	K	polar	positive	−3.9
Methionine	Met	M	nonpolar	neutral	1.9
Phenylalanine	Phe	F	nonpolar	neutral	2.8
Proline	Pro	P	nonpolar	neutral	−1.6
Serine	Ser	S	polar	neutral	−0.8
Threonine	Thr	T	polar	neutral	−0.7
Tryptophan	Trp	W	nonpolar	neutral	−0.9
Tyrosine	Tyr	Y	polar	neutral	−1.3
Valine	Val	V	nonpolar	neutral	4.2

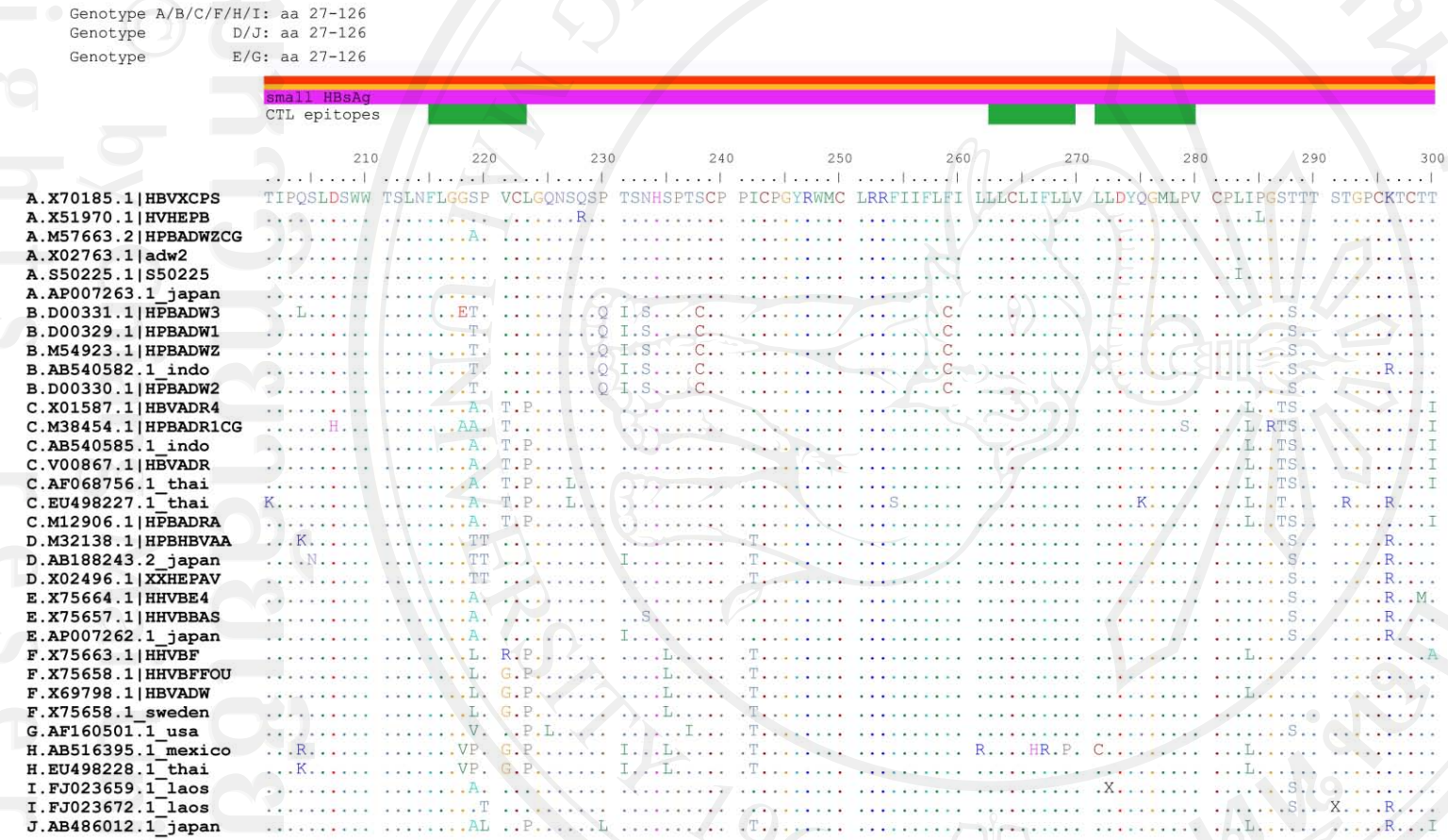
[Source: http://en.wikipedia.org/wiki/Amino_acid]

APPENDIX B

Multiple alignment of nucleotide sequences of HBV *S* gene for genotypes A to J







APPENDIX C

Abstract in French

ANALYSE DES MUTANTS DU VIRUS DE L'HEPATITE B (VHB) CHEZ LES PATIENTS CO-INFECTES PAR LE VIH ET LE VHB EN THAÏLANDE.

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RÉSUMÉ

L'infection par le VHB est endémique en Thaïlande (prévalence de l'AgHBs supérieure à 8%). Malgré l'efficacité du vaccin contre le VHB, la transmission périnatale reste une cause majeure d'infection chronique. Cette étude vise à identifier les mutants du VHB pouvant être associés à des échecs de vaccination, de diagnostic et de thérapeutique. Dans la première partie, nous avons analysé la prévalence de la transmission périnatale du VHB dans une cohorte issue d'un protocole thérapeutique de prévention de la transmission materno-fœtale du VIH. Nous avons cherché à caractériser les mutants d'échappement à la vaccination contre le VHB. Parmi 3349 femmes enceintes séropositives pour le VIH, l'antigène (Ag) HBs était positif dans 237 cas (7%) avec la technique ETI-MAK-4, Diasorin. L'AgHBs et/ou l'ADN du VHB étai(en)t positif(s) entre 2 et 6 mois d'âge chez 11 des 229 enfants nés de mères AgHBs positif. Les variants du VHB présents au sein de 9 de ces paires mère-enfant ont pu être étudiés après séquençage et clonage. Trois types de transmission ont pu être décrites : i) transmission de variants non mutés par les mères présentant

une charge virale VHB élevée (>6.5 log_{10}IU/mL), ii) transmission de variants minoritaires chez la mère (sK122R, sI126T), ii) transmission de mutants déjà présents à plus de 20% chez la mère (SI126M+P127S, ST13N+M133T+T140I+S204R). La capacité *in vitro* de ces mutants à échapper à la réponse neutralisante anti-HBs sera étudiée en utilisant un modèle de pseudo-particules portant les mutations identifiées. Dans une seconde partie, nous avons selectionné une cohorte de femmes enceintes séropositives pour le VIH, pour lesquelles la présence isolée d'Ac anti-HBc a été détectée. Dans cette population, la présence de marqueurs d'infection occulte par le VHB a été recherchée. Nous avons également analysé les facteurs de risque associés à la présence isolée d'Ac anti-HBc et à la présence d'infection occulte par le VHB. Parmi 1682 femmes AgHBs négatif avec le test ETI-MAK-4 (Diasorin), 229 (14%) avaient des Ac anti-HBc isolés. L'ADN du VHB était détectable par PCR chez 50 d'entre-elles. Parmi les 177 échantillons anti-HBc positifs, 177 ont pu être testés pour la présence de l'AgHBs avec un autre kit (MonoLisa®HBsAg ultra) : 12 résultats (7%) se sont avérés discordants entre les deux techniques. L'analyse multivariée a montré que l'âge (>35 ans) (OR=1.8, p=0.03), le lieu de naissance (région Nord de la Thaïlande) (OR=1.8, p>0.001), le nombre de CD4 <200 cellules/mL (OR=2.8, p>0.001) et la trace d'un contact avec le VHC étaient indépendamment associés à la présence isolée d'Ac anti-HBc. Dans la dernière partie, nous avons évalué l'efficacité à un an et à long terme de la lamivudine (3TC) sur la réplication du VHB chez 30 patients co-infectés par le VIH et le VHB, recevant une thérapie antirétrovirale hautement active (300 mg par jour de 3TC). La quantification de l'ADN du VHB et de l'ARN du VIH, la numération des lymphocytes CD4+ et CD8+ ainsi que la mesure de l'activité des transaminases ont été réalisés à l'introduction du traitement, à 3 mois,

à 12 mois et à l'issu du suivi à long terme (médiane : 50 mois, IQR=32-65). La virémie VHB médiane était de 7.35 log10 IU/mL (IQR=5.55-8.07). Après 3 et 12 mois, la virémie médiane avait diminué de 3.86 et de 4.40 log10 IU/mL avec dans 53% et 67% des cas une négativation de la virémie VHB (<2.18 log10 IU/mL). Elle était négative chez tous les patients présentant initialement un AgHBe négatif. A l'issu du suivi à long terme, la virémie était négative chez 17 des 19 patients (89%). Le taux cumulé d'obtention d'une virémie négative après 1, 2, 3, 5 et 7 ans était respectivement de 95%, 91%, 84%, 84% et 64%. Sept patients étaient rechuteurs. Deux patients étaient infectés par des variants avec une triple mutation (rtV173L+rtL180M+rtM204I) et un patient par un virus possédant une seule mutation de résistance à la lamivudine (rtM204I). Nous avons donc montré que l'administration de multithérapie antirétrovirale contenant de la lamivudine induisait la suppression à long terme de la réplication du VHB. Chez les patients co-infectés par le VIH et le VHB, cette stratégie thérapeutique apporte donc un bénéfice dans les pays en voie de développement.

Mots-clés : Hépatite B, co-infection par le VIH, échec de vaccination, résistance à la lamivudine, infection occulte par le VHB

APPENDIX D

Publications and Presentations

Publications

- **Khamduang W**, Gaudy-Graffin C, Ngo-Giang-Huong N, Jourdain G, Moreau A, Luekamlung N, Halue G, Buranawanitchakorn Y, Kunkongkapan S, Buranabanjasatean S, Lallemant M, Sirirungsi W, Goudeau A, and the Program for HIV Prevention and Treatment (PHPT) group. Long-term hepatitis B virus (HBV) response to lamivudine-containing highly active antiretroviral therapy in HIV-HBV co-infected patients in Thailand. *PLoS ONE* 7(7), 2012: e42184. doi:10.1371/journal.pone.0042184 (Journal Impact factor 2011: 4.092)
- **Khamduang W,** Ngo-Giang-Huong N, Gaudy-Graffin C, Jourdain G, Suwankornsakul W, Jarupanich T, Chalermpolprapa V, Nanta S, Puarattana-aroonkorn N, Tonmat S, Lallemant M, Goudeau A, Sirirungsi W and the Program for HIV Prevention and Treatment (PHPT-2) group. Prevalence and factors associated with isolated antibody to hepatitis B core antigen and occult HBV infection in HIV-1 infected pregnant women in Thailand. *Clinical Infectious Diseases.* 2013 (Journal Impact factor 2011: 9.154) (Accepted)

Presentations

Oral presentations

- **Khamduang W,** Gaudy-Graffin C, Moreau A, Ngo-Giang-Huong N, Jourdain G, Lallemant M, Pipatnakulchai S, Putiyanun C, Kunkongkapan S, Wannarit P, Sirinontakan S, Ardong W, Sirirungsi W, Goudeau A. Transmission of Hepatitis

B virus (HBV) minor variants in children born to HBV/HIV co-infected mothers. 5th Dominique Dormont International Conference, Mother-to-child transmitted viral diseases: from transmission to children care, 26-28th March, 2009, Paris, France, *Retrovirology* 2009; 6 (Suppl.1):O9

- **Khamduang W**, Gaudy-Graffin C, Moreau A, Ngo-Giang-Huong N, Jourdain G, Lallemant M, Pipatnakulchai S, Putiyanun C, Kunkongkapan S, Wannarit P, Sirinontakan S, Ardong W, Sirirungsi W, Goudeau A. Hepatitis B virus (HBV) virological response to combination antiretroviral treatment includes lamivudine (3TC) in HIV/HBV co-infected individuals in Thailand. 10ème réunion du Réseau National Hépatites de l'ANRS, 21st - 22nd January, 2010, Paris, France.
- **Khamduang W.** Franco-Thai Highlight: Hepatitis B Vaccine Escape. The International Workshop on "Interdisciplinary Approach to the Management of HIV: A Model for other Infectious Diseases", 16th –18th March, 2011, Chiang Mai, Thailand.
- **Khamduang W,** Ngo-Giang-Huong N, Sirirungsi W, Chanta C, Karnchanamayul W, Ngampiyaskul C, Sirithadthamrong C, Hongsiriwon S, Kamonpakorn N, Watanayothin S, Jourdain G. Prevalence of hepatitis B virus (HBV) infection in infants born to HIV/HBV co-infected women and factors associated with vertical transmission of HBV. The 4th International Workshop on HIV Pediatrics. 20th – 21st July 2012, Washington DC, USA. *Reviews in Antiviral therapy & Infectious Diseases 2012*, 8: 20: O18

Poster presentations

- **Khamduang W**, Gaudy-Graffin C, Moreau A, Ngo-Giang-Huong N, Jourdain G, Lallemant M, Pipatnakulchai S, Putiyanun C, Kunkongkapan S, Wannarit P, Sirinontakan S, Ardong W, Sirirungsi W, Goudeau A. Transmission of Hepatitis B virus (HBV) minor variants in children born to HBV/HIV co-infected mothers. 12th National AIDS Conference, 27-29th May, 2009, Bangkok, Thailand. *(CP08)*
- **Khamduang W**, Gaudy-Graffin C, Moreau A, Ngo-Giang-Huong N, Jourdain G, Lallemant M, Pipatnakulchai S, Putiyanun C, Kunkongkapan S, Wannarit P, Sirinontakan S, Ardong W, Sirirungsi W, Goudeau A. Hepatitis B virus (HBV) virological response to combination antiretroviral treatment includes lamivudine (3TC) in HIV/HBV co-infected individuals in Thailand. International Meeting; The molecular biology of hepatitis B viruses, 30th August - 2nd September, 2009, Tours, France. *(P-20)*
- **Khamduang W**, Gaudy-Graffin C, Ngo-Giang-Huong N, Jourdain G, Lallemant M, Pipatnakulchai S, Putiyanun C, Kunkongkapan S, Wannarit P, Sirinontakan S, Ardong W, Sirirungsi W, Goudeau A. The low prevalence of occult Hepatitis B infection in HIV-1 infected pregnant women with antibody to hepatitis B core antigen alone in Thailand. 18th international AIDS conference, 18-23th July, 2010, Vienna, Austria. *(THPE0205)*
- **Khamduang W,** Gaudy-Graffin C, Ngo-Giang-Huong N, Jourdain G, Moreau A, Luekamlung N, Halue G, Buranawanitchakorn Y, Kunkongkapan S, Buranabanjasatean S, Sureau C, Lallemant M, Sirirungsi W, Goudeau A and the Program for HIV Prevention and Treatment (PHPT) group. Hepatitis B Escape Mutants in Infants Born to Human Immunodeficiency Virus (HIV)-infected

Mothers Co-infected with Hepatitis B Virus (HBV). The 21st Conference of the Asian Pacific Association for the study of the liver (APASL), 17-20th February, 2011, Bangkok, Thailand. *(PP06.41)*

- **Khamduang W**, Gaudy-Graffin C, Ngo-Giang-Huong N, Jourdain G, Moreau A, Luekamlung N, Halue G, Buranawanitchakorn Y, Kunkongkapan S, Buranabanjasatean S, Lallemant M, Sirirungsi W, Goudeau A, and the Program for HIV Prevention and Treatment (PHPT) group. Long-term virological response of Hepatitis B virus to lamivudine-containing HAART in patients co-infected with HIV and HBV in Thailand. The 13th Thai national AIDS seminar, 29th – 31st March, 2011, Bangkok, Thailand. *(AP3)*
- **Khamduang W,** Gaudy-Graffin C, Ngo-Giang-Huong N, Jourdain G, Moreau A, Luekamlung N, Halue G, Buranawanitchakorn Y, Kunkongkapan S, Buranabanjasatean S, Lallemant M, Sirirungsi W, Goudeau A, for the Program for HIV Prevention and Treatment (PHPT) study group. Long-term virological response of Hepatitis B virus (HBV) to lamivudine-containing HAART in patients co-infected with HIV and HBV in Thailand. The XIX international AIDS conference, 22nd - 27th July 2012, Washington DC, USA. *(WEPE049)*

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PLoS one

Long-Term Hepatitis B Virus (HBV) Response to Lamivudine-Containing Highly Active Antiretroviral Therapy in HIV-HBV Co-Infected Patients in Thailand

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Abstract

Background: Approximately 4 million of people are co-infected with HIV and Hepatitis B virus (HBV). In resource-limited settings, the majority of HIV-infected patients initiate first-line highly active antiretroviral therapy containing lamivudine (3TC-containing-HAART) and long-term virological response of HBV to lamivudine-containing HAART in co-infected patients is not well known.

Methodology/Principal Finding: HIV-HBV co-infected patients enrolled in the PHPT cohort (ClinicalTrials.gov NCT00433030) and initiating a 3TC-containing-HAART regimen were included. HBV-DNA, HIV-RNA, CD4+ T-cell counts and alanine transaminase were measured at baseline, 3 months, 12 months and then every 6 months up to 5 years. Kaplan-Meier analysis was used to estimate the cumulative rates of patients who achieved and maintained HBV-DNA suppression. Of 30 co-infected patients, 19 were positive for HBe antigen (HBeAg). At initiation of 3TC-containing-HAART, median HBV DNA and HIV RNA levels were 7.35 $\log_{10}$ IU/mL and 4.47 $\log_{10}$ copies/mL, respectively. At 12 months, 67% of patients achieved HBV DNA suppression: 100% of HBeAg-negative patients and 47% of HBeAg-positive. Seventy-three percent of patients had HIV RNA below 50 copies/mL. The cumulative rates of maintained HBV-DNA suppression among the 23 patients who achieved HBV-DNA suppression were 91%, 87%, and 80% at 1, 2, and 4 years respectively. Of 17 patients who maintained HBV-DNA suppression while still on 3TC, 4 (24%) lost HBsAg and 7 of 8 (88%) HBeAg-positive patients lost HBeAg at their last visit (median duration, 59 months). HBV breakthrough was observed only in HBeAg-positive patients and 6 of 7 patients presenting HBV breakthrough had the rtM204I/V mutations associated with 3TC resistance along with rtL180M and/or rtV173L.

Conclusions: All HBeAg-negative patients and 63% of HBeAg-positive HIV-HBV co-infected patients achieved long-term HBV DNA suppression while on 3TC-containing-HAART. This study provides information useful for the management of co-infected patients in resource-limited countries where the vast majority of co-infected patients are currently receiving 3TC.

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Introduction

In 2010, the World Health Organization (WHO) estimated that 34 million people were HIV infected worldwide [1]; of whom, approximately 4 million have chronic hepatitis B virus (HBV) infection (defined by more than 6 months of hepatitis B surface antigen or HBsAg in the blood) [2]. These HIV-HBV co-infected individuals are at risk of accelerated liver disease progression, aggressive hepatocellular carcinoma and 8-fold increased liver-related mortality rate [3,4].

Current guidelines for the use of antiretroviral agents in HIV-1-infected patients [5,6,7], recommend to screen HBsAg in all HIV

patients prior to initiating antiretroviral treatment (ART), and if found positive, initiate combination antiretroviral with a tenofovir disoproxil fumarate (TDF) plus lamivudine (3TC) or emtricitabine (FTC) backbone. However, since HBsAg testing has not been available or is too costly in many resource-limited settings, HBV infection status is unknown for the vast majority of HIV infected patients who have thus received a standard first line that includes 3TC as part of triple combination antiretroviral therapy.

Lamivudine is a cytidine analogue that inhibits the reverse transcriptase of both HIV and HBV [8,9,10]. However, its major drawback is the progressive emergence of resistance mutations at a rate of 15–20% per year in HBV-HIV-1 co-infected patients in developed countries [11,12]. New generation nucleos(t)ide analogues with higher resistance barriers have been produced, in particular TDF for which no resistance mutation has been detected in chronically HBV infected patients after 3 years of therapy [13]. However, in resource-limited countries, most of these new drugs are not available or too expensive. Indeed, the 2008 Asian Pacific Association for the Study of the Liver (APASL), still recommends 3TC for treatment of HBV mono-infection in endemic areas [14].

In Thailand, since the national scale-up of ART in 2004 [15], over 95% of HIV-infected patients have received 3TC as part of highly active antiretroviral therapy (HAART) [16] and there are still numerous HIV-HBV co-infected patients on 3TC-containing HAART regimens for whom the long-term benefit of 3TC on HBV replication and the incidence of 3TC resistance mutations are not well known. We report the analysis of virological efficacy of 3TC on HBV replication and emergence of 3TC resistance HBV variants in HBV-HIV-coinfected patients receiving up to 5 years of 3TC-containing HAART regimens.

Methods

Patients

Patients were enrolled in the prospective multicenter Program for HIV Prevention and Treatment (PHPT) cohort (ClinicalTrials.gov Identifier: NCT00433030) of HIV-infected adults on antiretroviral therapy in Thailand and provided written informed consent at entry. This cohort study was approved by the Thai Ministry of Public Health and ethic committees at Chiangrai Prachanukroh, Prapokklao, Chonburi, Bhuddasothorn, Somdej Prapinklao, Nopparat Rajathanee, Bhumibol Adulyadej, Buddhachinaraj, Hat Yai, Samutsakorn, Nakhonpathom, Maharaj Nakornratchasrima, Sanpatong Hospitals. All investigators conducted the study according to the principles expressed in the Declaration of Helsinki. This sub-study was also approved by the ethic committee of the Faculty of Associated Medical Sciences, Chiang Mai University.

At entry, prior to starting HAART, all patients were screened for HBsAg and anti-HCV antibodies and had CD4+ T-cell count and plasma HIV RNA load performed. Following HAART initiation, CD4+ T-cell enumeration and plasma HIV RNA were measured every 6 months. Patients received a quarterly clinical biological follow-up and compliance was assessed at each visit by pill count and self-reporting.

Patients were included in this analysis if 1) HBsAg positive, 2) receiving HAART regimens which included 3TC (150 mg twice a day), 3) stored blood samples collected prior to 3TC initiation (baseline), and on HAART (3-month, 12-month, annual sample until up to 5 years) were available, and 4) HBV DNA was detectable at baseline.

HBV Markers and HIV RNA Quantification

HBsAg and HBeAg were tested at baseline using DiaSorin ETI-MAK-4 and DiaSorin ETI-EBK PLUS (Salluggia, Italy), respectively. HBsAg was tested in patients on 3TC treatment using the MonoLisa® HBsAg ultra with a sensitivity of 50 pg/mL (Bio-Rad laboratories, France). HBV viral load was quantified using the Abbott real-time HBV DNA^{TM} assay, Rungis, France (lower limit of detection 1.18 $\log_{10}$ IU/mL). HIV RNA was quantified using the COBAS Amplicor HIV-1 Monitor Test v.1.5. (Roche Molecular Systems, Branchburg, NJ) (lower limit of detection: 1.70 $\log_{10}$ copies/mL) and the Abbott real-time HIV RNA^{TM} assay, Abbott, (lower limit of detection: 1.6 $\log_{10}$ copies/mL).

HBV Virological Responses

HBV responses to 3TC were categorized according to the APASL recommendations [14]; HBV DNA suppression was defined as undetectable level of HBV DNA (the threshold used was 2.18 $\log_{10}$ IU/mL since some samples were diluted 1:10 due to insufficient volume); virological breakthrough defined as an initial decline >2 $\log_{10}$ IU/mL followed by an increase of HBV DNA >1 $\log_{10}$ IU; and maintained viral suppression defined as HBV DNA level persistently <2.18 $\log_{10}$ IU/mL.

HBV DNA Sequencing

All baseline samples as well as any sample with detectable HBV DNA on 3TC-containing-HAART were tested for HBV resistance mutation; HBV DNA sequencing was performed as previously described [17] with slight modification of PCR conditions. First round PCR conditions consisted of an initial 2 min denaturation step at 94°C, followed by 40 cycles of 1 min at 94°C for, 1 min at 48°C, and 3 min at 68°C. The second-round PCR included an initial denaturation step of 2 min at 94°C, followed by 30 cycles of 40 sec at 94°C, 1 min at 55°C, and 3 min at 68°C. The second-round PCR products were sequenced using the nested pol3M and pol4M primers and the BigDye Terminator Mix V. 1.1 (Applied Biosystems, Foster city, CA). Sequences were analyzed using the Bioedit software (http://www.mbio.ncsu.edu/bioedit) and HBV genotype was identified by phylogenetic analysis. HBV *pol* sequences were analyzed for polymorphisms and mutations known to be associated with 3TC resistance through comparison with wild-type reference sequences of similar genotype [18].

Statistical Analyses

Baseline characteristics are reported as percentage with 95% confidence interval (95%CI) or medians with interquartile ranges (IQR) and were compared according to HBeAg status using Fisher's exact test for categorical variables and Wilcoxon rank-sum test for continuous variables. Kaplan-Meier analysis was used to estimate the cumulative rates of achieving serum HBV DNA and, in those who achieved HBV DNA suppression, the duration of maintaining such suppression. Duration of HBV DNA suppression was counted from the date of first viral suppression until the date of first detectable HBV DNA or, if a patient was lost to follow-up or switched to a regimen without 3TC, the date of last undetectable HBV DNA load. Log-rank test was used to compare the cumulative rates of virological responses between HBeAg-positive and HBeAg-negative patients. For the analysis of association between the baseline CD4, HIV RNA, HBV DNA levels and virological responses to 3TC-containing HAART, continuous data were dichotomized according to the median baseline values and analysis was performed using Fisher's exact or log-rank tests. Statistical significance was defined as $p<0.05$. Data

were analyzed using STATA^{TM} version 10.1 software (Statacorp, College Station, Texas, USA).

Results

Baseline Characteristics

Of 1,448 HIV infected adults on HAART, 122 (8.4%) tested HBsAg-positive. Thirty patients met all criteria for this analysis i.e. receiving 150 mg twice a day (bid) of 3TC as part of HAART, blood samples collected at baseline and on 3TC-based treatment and detectable HBV DNA at baseline (Figure 1). Nineteen patients (63%) were HBeAg-positive. Patients' baseline characteristics were not different between HBeAg-positive and -negative patients (Table 1), except for the median HBV DNA level significantly higher, as expected, in HBeAg-positive patients and the alanine transaminase (ALT) level which tended to be higher in HBeAg-negative patients than in HBeAg-positive patients. Phylogenetic analysis indicated that 17% of patients were infected with HBV B genotype and 83% with C genotype (data not shown). None had HCV infection. Twelve patients (40%) had received previous antiretroviral treatment but none had been exposed to 3TC except one for a short period of 1 month. Over the course of 3TC-containing HAART, 29 of 30 patients had >95% adherence by pill count and 20 patients self-reported that they always missed ≤1 dose per week.

Efficacy of 3TC on HBV Replication

After 3 month of HAART, median reduction of HBV DNA was 3.86 $\log_{10}$ (IQR, 2.56–4.67), and 47% (95%CI, 28–66) of patients achieved HBV DNA suppression. At 12 months, median HBV DNA reduction was 4.40 $\log_{10}$ (IQR, 2.89–5.65) IU/mL and 20 patients (67%; 95%CI, 47–83) of patients achieved HBV DNA suppression.

The rate of HBV DNA suppression at 12 months was significantly higher among HBeAg-negative patients than among HBeAg-positive patients (100% and 47%, respectively; p = 0.004; Table 2 and Figure 2). Kaplan-Meier analysis showed that HBeAg-negative patients achieved HBV DNA suppression more rapidly than HBeAg-positive patients (p = 0.01). Six patients had partial HBV virological response and 4 experienced a HBV breakthrough during the first 12 months; of whom 2 had HBV DNA suppression at 3 months and 2 never fully suppressed HBV replication.

Over 5 years of follow-up, 17 of 20 patients who achieved HBV DNA suppression at 12 months were able to control their HBV replication until their last medical visit (with a median of 59 months [IQR, 32–60]), 2 had a viral breakthrough and one had no long-term sample available.

Of 6 patients with partial HBV virological response at 12 months, one patient achieved slower HBV DNA suppression, at 38 months of treatment, 3 had detectable HBV DNA (i.e. 2.71, 2.84, and 4.76 $\log_{10}$ IU/mL), one had a viral breakthrough at 24 months of treatment, and one switched treatment regimen (Table S1).

To analyze the relation between baseline HIV RNA load and HBV virological response to 3TC-containing HAART, patient baseline HIV RNA levels were dichotomized according to the median baseline HIV RNA level (4.47 $\log_{10}$ copies/mL). The baseline HBV DNA levels did not differ between the two baseline HIV RNA groups (7.19 versus 7.42 $\log_{10}$ IU/mL, p = 0.31). At one year, there was no difference in HBV response (67% versus 67%) by baseline HIV RNA group. Furthermore, there was no difference in response during 5-years of 3TC-containing HAART (log-rank p-value = 0.26).

The duration of HBV DNA suppression was analyzed for the 23 patients who achieved HBV DNA suppression (i.e. 20 achieved HBV DNA suppression at 12 months, 2 had a viral breakthrough

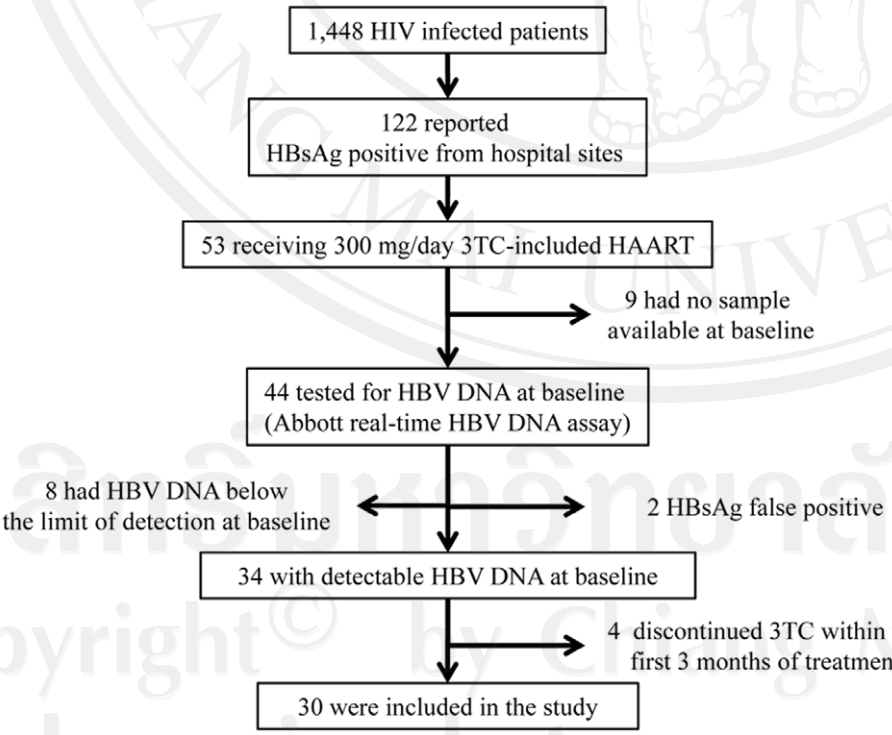

Figure 1. Disposition of patients included in this study.
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Table 1. Baseline demographic and clinical characteristics of the study population.

Baseline characteristics	Overall		HBeAg positive (N = 19)		HBeAg negative (N = 11)		p-value[a]
	n	Value	n	Value	n	Value	
Age (year) [median (IQR)]	30	31 (27–34)	19	29 (27–33)	11	33 (27–35)	0.59
Female [n (%)]	30	24 (80)	19	17 (89)	11	7 (64)	0.16
Treatment-experienced [n (%)]	30	12 (40)	19	7 (37)	11	5 (45)	0.71
CD4+ T-cell count (x10^6/L) [median (IQR)]	30	100 (38–178)	19	110 (38–188)	11	48 (33–178)	0.78
HIV RNA (log_{10}copies/mL) [median (IQR)]	30	4.47 (4.09–5.27)	19	4.46 (4.06–5.25)	11	5.25 (4.25–5.50)	0.29
Alanine transaminase (IU/L) [median (IQR)]	30	30 (20–39)	19	27 (17–36)	11	44 (21–121)	0.06
HBV DNA (log_{10}IU/mL) [median (IQR)]	30	7.35 (5.55–8.07)	19	7.92 (7.34–8.31)	11	3.76 (3.28–6.67)	<0.001
HBV Genotype B : C [n (%)]	30	5:25 (17:83)	19	4:15 (21:79)	11	1:10 (9:91)	0.63

[a]Fisher's exact test or Wilcoxon rank-sum test were used.
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within 12 months and one who achieved viral suppression after 12 months of treatment). Eighteen (78%) maintained HBV DNA suppression up to their last visit, 4 (17%) had a HBV breakthrough, and one changed drug regimen after 15 months of 3TC treatment (Table S1). The cumulative rates of maintained HBV DNA suppression were estimated to be 91% (95%CI; 69–98), 87% (95%CI; 64–95), 80% (95%CI; 55–92) and 80% (95%CI; 55–92) at 1, 2, 3, and 4 years, respectively (Figure 3).

HBV Serological Evaluations in Patients on 3TC-contained HAART

Of the 18 patients who maintained long-term HBV DNA suppression, 17 had a sample available for HBsAg testing. Four patients (24%) lost HBsAg at their last visit; one was HBeAg positive and three were HBeAg negative when initiating of 3TC-based HAART. Among the 8 HBeAg positive patients, 7 lost HBeAg at their last visit.

3TC Resistance-associated Mutations

There was no mutation associated with resistance to 3TC observed in all 30 patients before initiation of 3TC-containing-HAART. The 5-year cumulative rate of 3TC resistance was 20% (6/30); all of which was found in HBeAg positive patients. The mutations were found in 6 of 7 patients with viral breakthrough. Early viral breakthrough (within 1 year of therapy) occurred between 4 and 12 months in 4 patients. One patients had the HBV mutation ntG741A (G to A nucleotide at position 741), resulting in the known 3TC-associated resistance mutation rtM204I. This mutation led to the concomitant substitution of tryptophan to stop codon at position 196 (sW196stop) in the surface protein. The ntT843G mutation of unknown significance was also observed and resulted in the change from asparagine to lysine in the reverse transcriptase protein (rtN238K). In the 3 other patients, the viral breakthrough was not associated with the detection of HBV resistance mutations; however the M204V mutation was detected later during the follow-up in 2 patients, (i) at 42 months, along the compensatory resistance mutations rtV173L+L180M in one patient, and (ii) at 78 months with rtL180M mutation in one patient. No long-term HBV sequence was available for the third patient due to subsequent switch to non-3TC including regimen.

Late HBV breakthrough occurred in 3 patients and was associated with the emergence of 3TC-resistance mutations: rtV173L+L180M+M204I, rtL180M+M204V and rtL180M+M204I. Four patients never achieved undetectable

Table 2. HBV and HIV response to 3TC in HIV-1/HBV co-infected patients during 12 months of treatment.

	Overall (N = 30)		HBeAg positive (N = 19)		HBeAg negative (N = 11)		p-value[a]
	n	%[95%CI] or median [IQR]	n	%[95%CI] or median [IQR]	n	%[95%CI] or median [IQR]	
HBV DNA suppression[b]							
at 3 months	14	47 [28–66]	6	32 [13–57]	8	73 [39–94]	0.06
at 12 months	20	67 [47–83]	9	47 [24–71]	11	100 [72–100]	0.004
HIV load ≤50 cp/mL							
at 3 months	22	73 [54–88]	13	68 [43–87]	9	82 [48–98]	0.67
at 12 months	22	73 [54–88]	14	74 [49–91]	8	73 [39–94]	1.00
HIV RNA reduction							
at 3 months (log_{10} cp/mL)		2.92 [2.54–3.53]		2.93 [2.14–3.48]		2.91 [2.54–4.08]	0.53
at 12 months (log_{10} cp/mL)		2.92 [1.52–3.45]		2.93 [1.52–3.32]		2.91 [1.17–4.08]	0.78

[a]Fisher's exact test or Wilcoxon rank-sum test were used.
[b]HBV DNA suppression was defined as serum HBV DNA level equal or below 150 or 2.18 log_{10} IU/mL.
doi:10.1371/journal.pone.0042184.t002

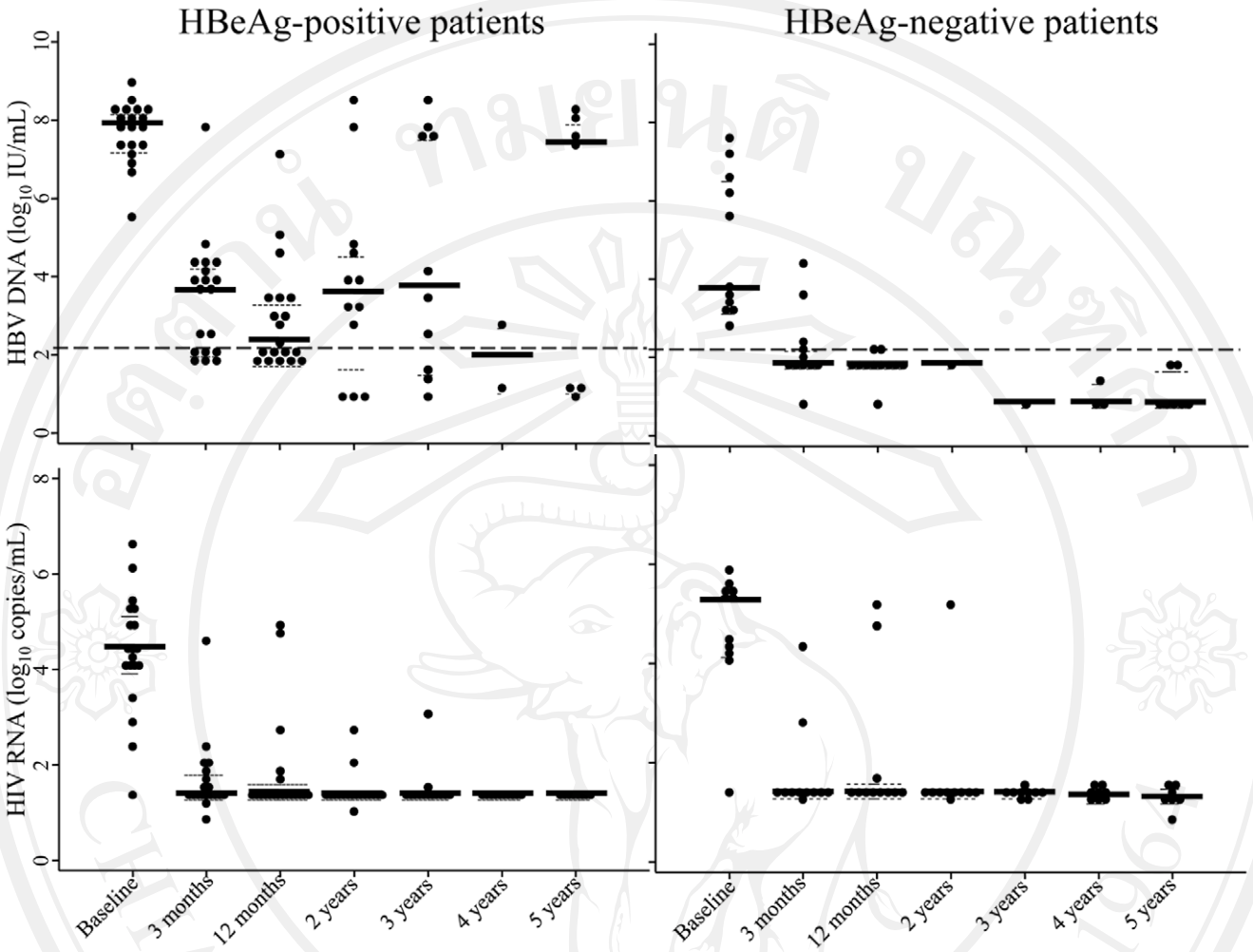

Figure 2. HBV DNA and HIV RNA load in HIV-HBV co-infected patients after initiation of 3TC-containing-HAART. Dotted line indicates the lower limit of detection for HBV DNA (2.18 log_{10} IU/mL).
doi:10.1371/journal.pone.0042184.g002

HBV DNA but no resistance mutations were found before they switched to non-3TC regimen or recieved add-on TDF.

Efficacy of HAART on HIV Replication, CD4 Cell Count and Alanine Transaminase Levels

At 3-month, the median reduction of HIV RNA was 2.92 log_{10} (IQR, 2.54–3.53) and 73% patients achieved undetectable HIV RNA load (<1.7 log_{10} or 50 copies/mL). At 12-month, the median HIV RNA reduction was 2.92 log_{10} (IQR, 1.52–3.45) copies/mL and 73% patients achieved undetectable HIV RNA load. Reduction of HIV RNA levels and proportions of undetectable HIV RNA were similar irrespective of baseline HBV DNA level and HBeAg status (Table 2). Six patients had HIV RNA load above 500 copies/mL and all presented the M184I/V mutations associated with HIV resistance to 3TC. Median CD4+ T-cell counts increased from 100 (IQR: 38–178)$\times 10^6$ cells/L at baseline to 247 (IQR: 197–374) at 12-month and 472 x10^6 cells/L at 5 years of treatment, respectively. Median ALT was 30 (IQR: 20–39) IU/L and decreased but not significantly to 21 IU/L (IQR: 16–29) at 5 years of 3TC treatment. Kinetics of CD4+ T-cell counts and serum ALT levels in HIV-HBV co-infected patients after initiation of 3TC-containing HAART are showed in Figure 4, according to their HBeAg status. Patients with baseline CD4 T-cell counts above the median (100×10^6 cells/L) had similar rates of undetectable levels of both HBV DNA and HIV RNA over 5 years of 3TC-containing HAART as those with CD4 T-cell counts below the median (log-rank p-values: 0.42 for HBV and 0.41 for HIV). Also, over 5-years of 3TC-containing HAART, there was no difference in increase of absolute CD4+ T-cells count among HBV virological responders and non-responders.

Discussion

We analyzed the long-term HBV and HIV virological response in a group of 30 HIV-HBV co-infected patients, 63% HBeAg-positive, who received 3TC, as part of HAART regimen. After 12 months of 3TC-containing-HAART, the rate of HBV DNA suppression in our study was 67%, compared to the 40% reported in the international collaborative (CAESAR) study conducted in Canada, Australia, Europe and Africa [8] despite higher median HBV DNA level prior to 3TC initiation, 7.35 vs. 6.87 log_{10} IU/mL. A recent study conducted in Kenya [19] reported that 89% (17 of 19) of HIV-HBV co-infected patients achieved HBV DNA suppression (<100 IU/mL) after 18 months of 3TC treatment. The rate of HBV DNA suppression among HBeAg negative patients was 94% (17 of 18) similar to the 100% rate observed in

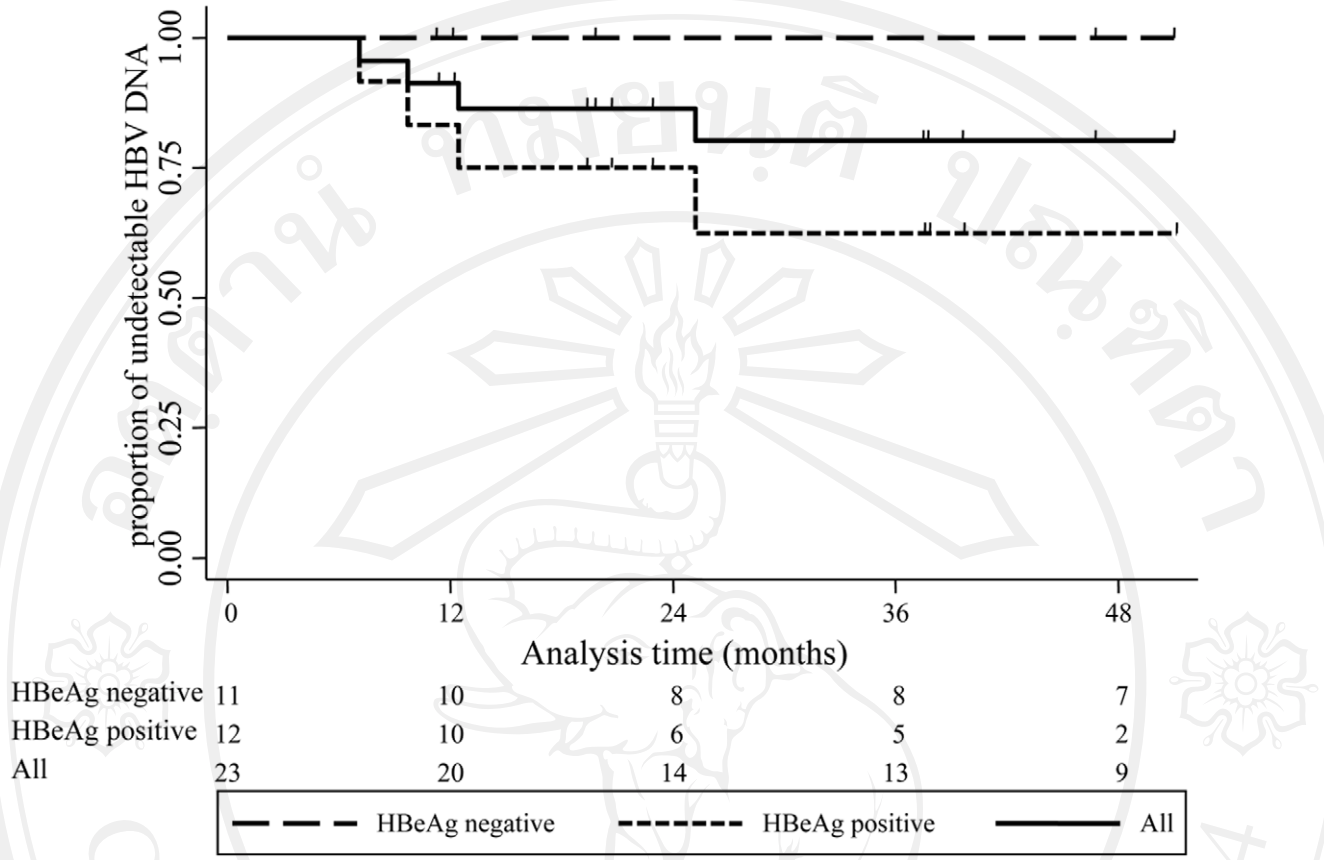

Figure 3. Kaplan-Meier curve of time to loss of HBV DNA suppression in 23 HIV-HBV co-infected patients who had achieved HBV DNA suppression during the course of 3TC-containing-HAART.
doi:10.1371/journal.pone.0042184.g003

our study. In contrast, the recent PHIDISA II study conducted among 57 HIV-HBV co-infected patients in South Africa [20] showed that HBV DNA suppression rate slight increased from 7% at baseline to 34% at 3 months of 3TC treatment and remained stable until 12 months. In the PHIDISA II study, the baseline HBV DNA level was 7.00 $\log_{10}$ IU/mL and similar to the level in our study. Furthermore, we found no relation between the baseline HIV RNA level and HBV response to 3TC-containing HAART or between the baseline HBV DNA level and HIV response to 3TC-containing HAART. Baseline CD4 count was not associated with either HBV or HIV virological response. In addition, over 5-years of 3TC-containing HAART, increases of absolute CD4+ T-cells count were not different among HBV virological responders and non-responders.

Among the 23 patients who had achieved HBV DNA suppression, 18 (78%) maintained HBV DNA suppression over a median of 51 months. This rate is much higher than the 9% reported by Benhamou et al, in HIV-HBV co-infected patients after 4 years of treatment with the same dosing of 3TC [11]. Of interest the threshold used to define HBV DNA suppression was 4.03 $\log_{10}$ IU/mL while it was 2.18 $\log_{10}$ IU/mL in our study. The higher response rate in our study may be due to a better compliance of patients to their treatment as evidenced by 73% of patients achieving HIV RNA below 50 copies/mL at 12 months and most of patients had undetectable HIV RNA, except some with high replication of drug-resistant strains. Another possibility could be that the HBV genotypes B and C are more sensitive to 3TC than genotypes A and D, which require confirmation with *in vitro* experiments. HBV DNA suppression was maintained in all HBeAg-negative patients. The higher rate of response to 3TC treatment and duration of HBV DNA suppression among HBeAg-negative patients suggest that, in resource-limited countries, HBeAg testing may be a valuable marker to predict the virological response to 3TC and could be considered when initiating in HIV-HBV co-infected patients the first-line HAART which usually includes 3TC. Other studies conducted among patients with chronic HBV infection in Japan and Brazil showed that HBeAg-negative patients had better HBV response to 3TC [21,22].

HBeAg seroconversion and HBsAg loss are usually associated with favorable clinical outcome. Twenty-four percent (4/17) of patients lost HBsAg and 88% (7/8) of HBeAg-positive patients lost HBeAg at their last visit (median duration of 59 months). Although our study had a limited number of patients, our results are consistent with those reported in a recent study conducted in Taiwan among HBV-HIV infected patients receiving 3TC-containing HAART (median duration of 34 months), 6% (6/108) of HBsAg loss and 46% (21/46) of HBeAg seroconversion [23].

One major limitation of treating HBV with 3TC monotherapy is the rapid emergence of resistance mutations. In HBV-HIV-1 co-infected patients, resistance mutations to 3TC have been shown to occur at a rate of 15–20% per year [11,24]. In our study, the incidence of 3TC resistance mutations detected during the first year of therapy was 3% (1of 30) which is comparable to the 7% (2 of 27) in a study conducted in Kenya ($p = 0.60$) [19]. Over the 5 years of follow-up, 6 of 7 patients presenting HBV breakthrough

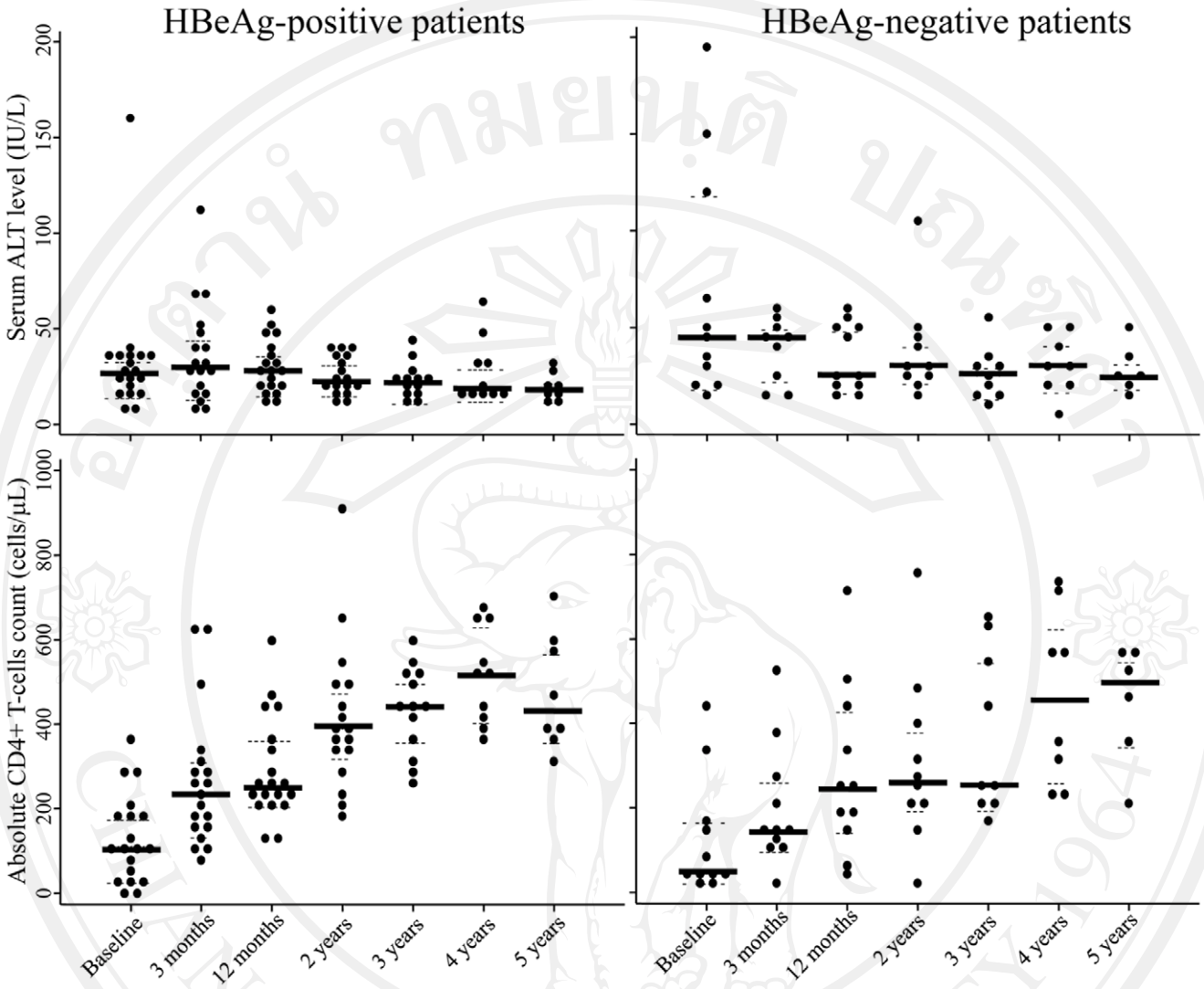

Figure 4. Serum ALT level and CD4+ T-cells count in HIV-HBV co-infected patients after initiation of 3TC-containing-HAART.
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had the rtM204I (due to the ntG741A uncommon mutation) or M204V mutations associated with 3TC resistance along with rtL180M and/or rtV173L. Despite good compliance to treatment, as evidenced by the HIV RNA suppression, some patients had experienced HBV breakthrough without any mutations within the *pol* gene. This may be due to the emergence of mutations outside the *rt* domain or other mechanisms yet to identified.

A nucleotide analogue, TDF, has been shown to be active against both wild-type and 3TC resistant HBV [25,26]. It has recently been available in Thailand at the price of 38 USD per month, which exceeded that of the current standard first line HAART (zidovudine/stavudine, 3TC and nevirapine), 30 USD per month [27,28]. Although the Thai national [29], US [6] and WHO guidelines [30], have recommended the use of TDF+3TC or TDF+FTC as the backbone of HAART combination to treat HIV-HBV co-infected patients, this combination may not be provided to all HIV-HBV co-infected patients. Indeed, identifying HBV co-infected patients still poses a problem as shown in the study of Sungkanuparph et al where about 42% of Thai HIV-1 infected patients on ART had not been assessed for HBV co-infection [31].

In conclusion, our study shows that all HBeAg negative patients and a significant number of HBeAg positive HIV-HBV co-infected patients can achieve long-term HBV DNA and HIV suppression on 3TC containing HAART. The results of this study provide further information which may be useful for the management of HIV-HBV co-infected patients in resource-limited countries where the vast majority of HIV-HBV co-infected patients are currently receiving 3TC.

Supporting Information

Table S1 Summary of HBV DNA and HIV RNA loads of HIV-HBV co-infected patients on lamivudine-containing HAART.
(DOCX)

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Author Contributions

Conceived and designed the experiments: WK CG NN WS AG. Performed the experiments: WK AM. Analyzed the data: WK CG NN WS AG. Contributed reagents/materials/analysis tools: CG NN WS AG GJ ML. Wrote the paper: WK CG NN AG. Responsibility for patient recruitment and treatment: NL GH YB SK SB. Supervised the study: GJ ML.

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MAJOR ARTICLE

Prevalence, Risk Factors, and Impact of Isolated Antibody to Hepatitis B Core Antigen and Occult Hepatitis B Virus Infection in HIV-1–Infected Pregnant Women

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Background. Prevalence and risk factors for isolated antibody to hepatitis B core antigen (anti-HBc) and occult hepatitis B virus (HBV) infection are not well known in human immunodeficiency virus type 1 (HIV-1)–infected pregnant women. It is unclear if women with occult infections are at risk of transmitting HBV to their infants.

Methods. HIV-1–infected and HBV surface antigen (HBsAg)–negative pregnant women were tested for antibody to HBsAg (anti-HBs) and anti-HBc using enzyme immunoassay. Women with isolated anti-HBc were assessed for occult HBV infection, defined as HBV DNA levels >15 IU/mL, using the Abbott RealTime HBV DNA assay. Infants born to women with isolated anti-HBc and detectable HBV DNA were tested at 4 months of age for HBV DNA. Logistic regression analysis was used to identify factors associated with isolated anti-HBc and occult HBV infection.

Results. Among 1812 HIV-infected pregnant women, 1682 were HBsAg negative. Fourteen percent (95% confidence interval [CI], 12%–15%) of HBsAg-negative women had an isolated anti-HBc that was independently associated with low CD4 count, age >35 years, birth in northern Thailand, and positive anti–hepatitis C virus serology. Occult HBV infection was identified in 24% (95% CI, 18%–30%) of women with isolated anti-HBc, representing 2.6% (95% CI, 1.9%–3.5%) of HIV-1–infected pregnant women, and was inversely associated with HIV RNA levels. None of the women with isolated anti-HBc and occult HBV infection transmitted HBV to their infants.

Conclusions. HIV-1–infected pregnant women with isolated anti-HBc and occult HBV infection have very low HBV DNA levels and are thus at very low risk to transmit HBV to their infants.

Keywords. HIV-1–infected pregnant women; isolated anti-HBc; occult HBV infection; perinatal transmission.

Over the past decade, individuals with isolated antibody to hepatitis B core antigen (anti-HBc) serology (ie, anti-HBc positive in the absence of detectable hepatitis B surface antigen [HBsAg] and hepatitis B surface antibody [anti-HBs]) have drawn much attention to their management because they are potentially infectious. Indeed, transmission of hepatitis B virus (HBV) from isolated anti-HBc individuals has been reported following sexual contact [1], blood transfusion [2], organ transplantation [3], or during the perinatal

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[a]The members of the PHPT-2 group are listed in the Notes section.

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period [4]. Furthermore, immunosuppressed patients with isolated anti-HBc can reactivate HBV replication following therapies or infectious diseases [5]. The clinical significance of this serology pattern remains unclear. Although the majority of individuals with this serology pattern present with normal liver enzyme levels and no sign of liver disease, it has been observed in patients presenting with cirrhosis and hepatocellular carcinoma [6, 7] and has been associated with a significantly shorter survival in patients infected with human immunodeficiency virus (HIV) as compared to those with anti-HBs antibody [8]. Moreover, the Multicenter AIDS Cohort Study reported a 3.6-fold increased risk of non-AIDS-related deaths in HIV-infected men with isolated anti-HBc compared to those without HBV infection [9]. The prevalence of isolated anti-HBc varies according to that of chronic HBV infection in the population; the rate among blood donors ranges between 1% and 4% [1, 10] in countries with low chronic HBV infection prevalence and between 1%–21% in high chronic HBV infection prevalence countries [11–13]. Higher isolated anti-HBc prevalence, ranging between 17% and 81%, has been found among HIV-infected [14–18] or hepatitis C virus (HCV)–infected individuals [19] and injection drug users [18, 20]. Individuals with isolated anti-HBc represent a heterogeneous population that comprises individuals who had completely cleared HBV infection but lost their anti-HBs antibody and those with occult HBV infection defined by the presence of HBV DNA in their serum and/or liver without detectable HBsAg, irrespective of other HBV serological markers [21]. The latter may present higher risk of transmission and liver disease progression.

Among HIV-infected patients, prevalence of occult HBV infection ranges between 0% and 89%, with most studies reporting HBV DNA values of <1000 IU/mL [22], and is much higher among individuals with isolated anti-HBc [23]. However, there are still very limited data on occult HBV infection in HIV-infected pregnant women and its impact on HBV transmission to their infants.

In this study, we assessed the prevalence and risk factors for isolated anti-HBc and occult HBV infection among a large number of HIV type 1 (HIV-1)–infected pregnant women in Thailand and evaluated the risk of HBV transmission to their infants.

MATERIALS AND METHODS

Study Population

The study population was derived from HIV-1–infected pregnant women who participated between 2001 and 2003 in a clinical trial conducted in Thailand that investigated the efficacy of zidovudine plus single-dose nevirapine to prevent mother-to-child transmission of HIV-1 (PHPT-2 [ClinicalTrials.gov identifier: NCT00398684]) [24]. Demographic, clinical, and biological data were collected before enrollment and during the study. Maternal and infant blood samples were collected at entry and during study and plasma/sera were stored frozen. Only HBsAg-negative women were included in this study, which was approved by the Ethics Committee of the Faculty of Associated Medical Sciences, Chiang Mai University.

Analysis of HBV Infection Markers

Women were screened for HBsAg using an enzyme immunoassay of 250 pg/mL sensitivity (DiaSorin ETI-MAK-2, Salluggia, Italy). HBsAg-negative women were tested for anti-HBc (Monolisa anti-HBc PLUS) and anti-HBs (Monolisa anti-HBs PLUS, Bio-Rad Laboratories, Marnes La Coquette, France). Women positive for both anti-HBc and anti-HBs antibodies were considered as having resolved HBV infection; those with only anti-HBc were considered as having acquired HBV infection; those positive for anti-HBs only were considered as having received hepatitis B vaccine; and those negative for both anti-HBc and anti-HBs antibodies were considered as having not acquired HBV infection.

Women with isolated anti-HBc had HBV DNA quantified using the Abbott RealTime HBV DNA assay (Abbott France, Rungis, France; lower limit of detection of 15 IU/mL or 1.18 $\log_{10}$ IU/mL) and HBsAg verified using an HBsAg test kit of 50 pg/mL sensitivity (Monolisa HBsAg Ultra, Bio-Rad Laboratories) and able to detect up to 30 additional mutations on HBsAg proteins.

Infants born to women with isolated anti-HBc and detectable HBV DNA were tested at 4 months of age for HBV DNA using the Abbott RealTime HBV DNA assay. Children were followed up until 12 months of age.

HBV Sequencing

HBV sequencing was performed for women with detectable HBV DNA. HBV DNA was extracted from women's plasma using the automatic sample extraction system (Abbott M2000sp, Rungis, France). Ten microliters of HBV DNA extract was used as the template for nested polymerase chain reaction (PCR). Published primers were used to amplify HBV surface/polymerase region (nucleotide position 251 to 1058) [25]. Amplicons were sequenced using the BigDye Terminator Mix V. 1.1 (Applied Biosystems, Foster City, California) and the ABI PRISM 3100 Genetic Analyzer, and sequencing data were analyzed using the software Bioedit (version 7.0.9.0).

Statistical Analysis

Characteristics of women including age at enrollment, region of birth, prior pregnancies, alanine aminotransferase (ALT) level, white blood cells, lymphocytes, $CD4^+$ and $CD8^+$ T-cell counts, and the presence of antibodies against syphilis and hepatitis C virus were described using number and percentage for

categorical data and median with interquartile range (IQR) for continuous data. Univariate analyses were performed using logistic regression analysis to identify risk factors for having isolated anti-HBc or occult HBV infection. Continuous variables were transformed into categorical variables using common cutoff values. For multivariate analysis, all factors with a P value <.20 identified by univariate analysis were then introduced into the forward stepwise logistic regression model, to investigate independent risk factors associated with isolated anti-HBc serology or occult HBV infection. All data analyses were performed using Stata version 10.1 software (StataCorp, College Station, Texas). Differences were considered statistically significant if the P value was ≤.05.

RESULTS

Characteristics of Study Population

Of 1812 HIV-1–infected pregnant women, 1752 were found to be HBsAg-negative, of whom 1682 (96.0%) had sufficient samples to be included in this study (Figure 1). Baseline characteristics of HBsAg-negative women are described in Table 1. At enrollment, the median age was 26 years, median ALT level was normal, median HIV RNA load was 4.03 $\log_{10}$ copies/mL, and median $CD4^+$ and $CD8^+$ T-cell counts were 378 cells/μL and 915 cells/μL, respectively. One percent of women were anti–syphilis antibody positive and 5% were anti-HCV positive (Table 1). Women who were not included in this study because of insufficient samples had similar baseline characteristics: age of enrollment, ALT level, $CD4^+$ and $CD8^+$ T-cell counts, and HIV RNA load (data not shown).

HBV Serology Profile Among HBsAg-Negative, HIV-1–Infected Pregnant Women

Of 1682 HBsAg-negative women, 553 (33%; 95% CI, 31%–35%) had markers of resolved HBV infection (anti-HBc and anti-HBs positive), 229 (14%; 95% CI, 12%–15%) had marker of exposure to HBV (isolated anti-HBc), 68 (4%; 95% CI, 3%–5%) had marker of hepatitis B vaccine (anti-HBs positive only), and 832 (49%; 95% CI, 47%–52%) had no markers of exposure to HBV (anti-HBc and anti-HBs negative).

The prevalence of isolated anti-HBc antibodies differed according to the region of birth; the highest rate, 22%, was found in women born in the northern region of Thailand, whereas the lowest rate, 4%, was found in the southern region (Table 2). The rate of isolated anti-HBc in HIV-1–infected pregnant women increased with their age (Figure 2).

Factors Associated With Isolated Anti-HBc

Among all parameters analyzed, univariate analysis showed that age >35 years, birth in northern region, white blood cell count <7500 cells/μL, lymphocyte count <1000 cells/μL, $CD4^+$ T-cell count <350 cells/μL, and HCV infection were significantly associated with isolated anti-HBc serology in HIV-1–infected pregnant women (Table 3). After adjustment on all significant parameters, factors independently associated with isolated anti-HBc were age >35 years (adjusted odds ratio [AOR], 1.8; $P = .029$); birth in northern region (AOR, 1.8; $P < .001$); absolute $CD4^+$ cell count <350 cells/μL (AOR, 1.5; $P = .02$) and, much more significant, $CD4^+$ cell count <200 cells/μL (AOR, 2.8; $P < .001$); and exposure to HCV (AOR, 2.6; $P = .001$) (Table 3).

Prevalence of Occult HBV Infection Among HIV-1–Infected Pregnant Women With Isolated Anti-HBc

We first verified the absence of HBsAg in all women with isolated anti-HBc using a different HBsAg test kit. Of 228 women with available samples, 12 (5%) tested positive for HBsAg with the new test kit. Samples of all but 1 woman showed a low signal-to-cutoff ratio, ranging from 1.02 to 2.79 (median, 1.4 [IQR, 1.1–2.0]). Women with discrepant HBsAg results were then excluded from further analysis.

Among all 216 HIV-1–infected pregnant women with confirmed isolated anti-HBc serology, 200 had a sample available for HBV DNA quantification. All 200 women had HBV DNA <1000 IU/mL; 153 had HBV DNA below the limit of detection (15 IU/mL), 44 had HBV DNA level between 15–100 IU/mL, and 3 had HBV DNA between 101 and 1000 IU/mL. The prevalence of occult HBV infection among women with isolated anti-HBc was thus 23.5% (47/200; 95% CI, 18%–30%). Of 47 women with detectable HBV DNA (>15 IU/mL), 2 had successful HBV sequencing: one (16 IU/mL HBV DNA level) had sS117I, sT118K, and sR160K mutations (GenBank accession number: JX402002), and the other (117 IU/mL HBV DNA) had no S gene mutation (GenBank accession number: JX402003).

Factors Associated With Occult HBV Infection in HIV-1–Infected Pregnant Women With Isolated Anti-HBc Serology

Among all parameters analyzed, only HIV RNA level was inversely associated with occult HBV infection in HIV-1–infected pregnant women having isolated anti-HBc serological pattern (AOR, 0.2; $P = .013$; Table 4).

Assessment of HBV Infection in Infants Born to Mothers With Occult HBV Infection

We assessed HBV infection in infants born to 47 mothers with detectable HBV DNA (>15 IU/mL) at enrollment. No HBV DNA was detected in any of their infants at 4 months of age.

DISCUSSION

This is the first detailed analysis of HBV serologic markers among a large number of HIV-1–pregnant women. We have analyzed 3 variables associated with isolated anti-HBc profile

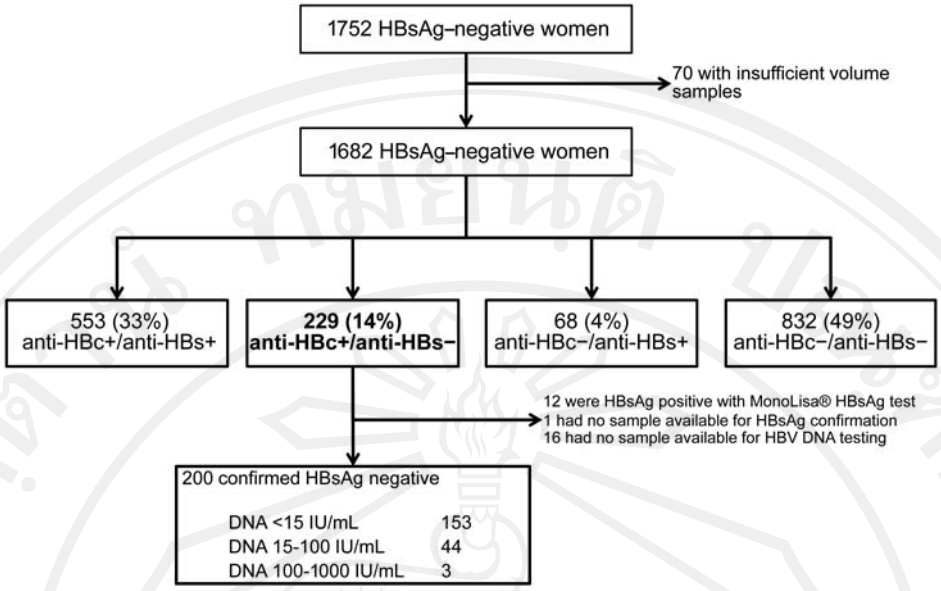

Figure 1. Overall study diagram. Abbreviations: anti-HBc, antibody to hepatitis B core antigen; anti-HBs, antibody to hepatitis B surface antigen; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen.

and occult HBV infection: prevalence, risk factors, and impact on perinatal transmission of HBV. Consistent with data from regions where vertical transmission of HBV has significant contribution, about half of HIV-1–infected pregnant women in our study showed HBV exposure markers. Fourteen percent had isolated anti-HBc. This rate is close to that observed among HIV-1–infected adults in Bangkok (20%) [20], northern areas (13%; S. Thongsawat, unpublished data), and other countries with high prevalence of chronic HBV infection [11–13].

Table 1. Characteristics of HIV-1–Infected Pregnant Women Negative for Hepatitis B Surface Antigen

Characteristic	No.	Median (IQR) or No. (%)
Age at enrollment, y	1682	25.9 (22.7–29.7)
Region of birth	1682	
Central		373 (22)
Eastern		256 (15)
Northern		348 (21)
Northeastern		553 (33)
Southern		76 (5)
Western		76 (5)
Prior pregnancy	1678	1041 (62)
SGPT or ALT, IU/L	1638	15 (10–24)
WBC count, cells/μL	1652	8615 (7300–10 160)
Absolute lymphocyte count, cells/μL	1650	1805 (1430–2250)
Absolute CD4 count, cells/μL	1671	378 (245–531)
Absolute CD8 count, cells/μL	1634	915 (700–1193)
HIV RNA load, $\log_{10}$ copies/mL	1660	4.03 (3.37–4.65)
Anti-syphilis antibody positive	1649	17 (1)
Anti-HCV antibody positive	1659	75 (5)

Abbreviations: ALT, alanine aminotransferase; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IQR, interquartile range; SGPT, serum glutamic pyruvic transaminas; WBC, white blood cell.

About half of HIV-1–infected pregnant women had no HBV serological markers, indicating they are an HBV-susceptible population. Because immunization with HBV vaccine is strongly recommended for all HIV-infected individuals without immunity to HBV [26], our finding highlights the need for testing of all HIV-infected patients in order to vaccinate uninfected people who are not immune.

We identified several independent risk factors for isolated anti-HBc serological status in HIV-1–infected pregnant women: low CD4 count, age >35 years, and HCV infection. These factors have also been found in other populations, both HIV-infected [8, 22, 27] and HIV-uninfected [28]. Severe immunocompromised status, CD4 T-cell count <100 cells/μL, has been associated with loss of anti-HBs and development of isolated anti-HBc in HIV-positive patients [22]. The effect of age may be related to the progressive loss of anti-HBs producing capacity over time after resolution of HBV infection, or insufficient level of anti-HBs production [29]. We also found that being born in the northern region of Thailand was independently associated with isolated anti-HBc. Other studies have reported higher prevalence of HBsAg positivity in the northern region of Thailand as compared to the southern region [30–32], which may explain the rates of isolated anti-HBc observed

Table 2. Hepatitis B Virus Serological Status According to Region of Birth of Hepatitis B Surface Antigen–Negative Women

Status	Central	Eastern	Northern	Northeastern	Southern	Western	Total
Anti-HBc+/anti-HBs+	128 (34)	74 (29)	148 (43)	161 (29)	20 (26)	22 (29)	553 (33)
Anti-HBc+/anti-HBs–	38 (10)	31 (12)	77 (22)	69 (12)	3 (4)	11 (14)	229 (14)
Anti-HBc–/anti-HBs+	26 (7)	10 (5)	16 (5)	10 (2)	1 (1)	5 (7)	68 (4)
Anti-HBc–/anti-HBs–	181 (49)	141 (55)	107 (31)	313 (57)	52 (68)	38 (50)	832 (49)

Abbreviations: anti-HBc,antibody to hepatitis B core antigen; anti-HBs, antibody to hepatitis B surface antigen.

in our study. Our results are also consistent with other studies describing HCV infection as a main factor for isolated anti-HBc in both HIV-infected and -uninfected populations [1, 14, 17, 19, 20, 33], possibly as a result of the direct interference of HCV core protein on the synthesis of HBsAg [34, 35].

A wide range (0%–89%) of occult HBV infection has been reported in HIV-infected patients with isolated anti-HBc [22]. The heterogeneity of study populations and the usage of different sensitivity and specificity of HBV DNA assays may account for these discrepancies. In this study, we used a highly sensitive commercial technique to detect HBV DNA and were thus able to detect HBV DNA in 24% (47 of 200) of HIV-1–infected pregnant women with isolated anti-HBc serological profile. These rates of occult HBV infection are within the range found in isolated anti-HBc blood donors (4%–24%) of high-HBV-endemic areas such as India, Taiwan, Japan, and Sardinia [36]. When considering the whole population of HIV-1–infected pregnant women, the prevalence of isolated anti-HBc and occult HBV infection was 2.6% (47/1783; 95% CI, 1.9%–3.5%).

One intriguing observation was the inverse association between the detection of HBV DNA and HIV RNA concentrations. Unlike Lo Re et al [37] who found more frequently occult HBV infection in patients with HIV RNA >1000 copies/mL (17% in patients with a high HIV RNA level vs 4.6% in those with a low HIV RNA level, n = 179), we observed a higher rate of occult HBV infection in patients (n = 200) with low HIV RNA concentrations than in those with high HIV RNA level (42% vs 21%; $P = .04$). Possible explanations to this could be that 73% of patients in Lo Re et al's study were on highly active antiretroviral treatment, whereas in our study all women were naive to antiretroviral treatment. Further studies are needed to understand this discrepancy.

The clinical relevance of isolated anti-HBc and impact of low levels of HBV DNA in HIV-pregnant women with isolated anti-HBc are not well known. Walz et al recently reported that 7 of 105 infants born to women with isolated anti-HBc were infected with HBV, but none of the infants were positive for both HBsAg and HBV DNA. Interestingly, only 1 woman was HBV DNA positive [38]. In our study, the level of HBV DNA was <1000 IU/mL in 47 women with occult HBV infection, and none transmitted HBV to their infants.

Our study has several limitations. First, we quantified HBV DNA at only 1 time point, which may be insufficient as HBV DNA levels can fluctuate over time, depending on the phase of infection and host immune responses. However, pregnant

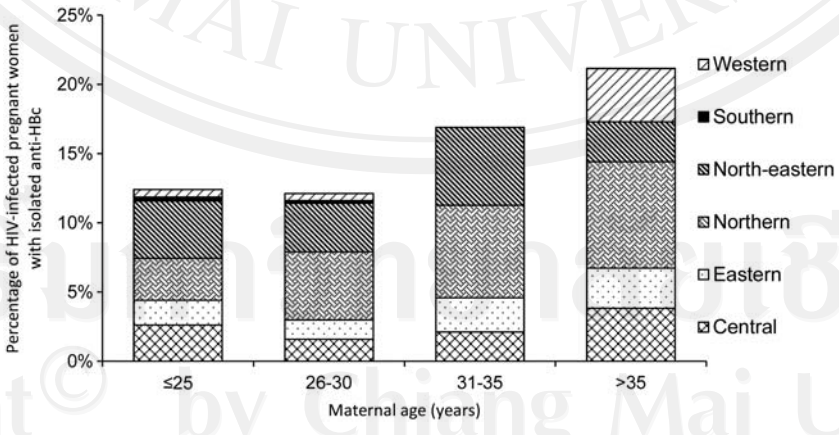

Figure 2. Age-related distribution of patients with isolated antibody to hepatitis B core antigen. Abbreviations: anti-HBc, antibody to hepatitis B core antigen; HIV, human immunodeficiency virus.

Table 3. Factors Associated With Isolated Antibody to Hepatitis B Core Antigen in HIV-1–Infected Pregnant Women

				Univariate Analysis		Multivariate Analysis	
Parameter	Category	No.	Isolated Anti-HBc (%)	OR (95% CI)	*P* Value[a]	OR (95% CI)	*P* Value[b]
Age at enrollment, y	≤25	725	90 (12)	1.0			
	26–30	569	69 (12)	1.0 (0.7–1.4)	.88		
	31–35	284	48 (17)	1.4 (1.0–2.1)	.06		
	>35	**104**	**22 (21)**	**1.9 (1.1–3.2)**	**.016**	**1.8 (1.1–2.9)**	**.029**
Region of birth	Central	373	38 (10)	1.0			
	Eastern	256	31 (12)	1.2 (.7–2.0)	.45		
	Northern	**348**	**77 (22)**	**2.5 (1.6–3.8)**	**<.001**	**1.8 (1.3–2.5)**	**<.001**
	Northeastern	553	69 (12)	1.3 (.8–1.9)	.29		
	Southern	76	3 (4)	.4 (.1–1.2)	.10	.4 (.1–1.1)	NS
	Western	76	11 (14)	1.5 (.7–3.1)	.28		
Prior pregnancy	No	637	83 (13)	1.0			
	Yes	1041	145 (14)	1.1 (.8–1.4)	.60		
ALT, IU/L	≤30	1437	190 (13)	1.0			
	31–60	168	25 (15)	1.1 (.7–1.8)	.55		
	>60	33	7 (21)	1.8 (.8–4.1)	.19		
WBC count, cells/μL	>10 000	440	44 (10)	1.0			
	7501–10 000	723	91 (13)	1.3 (.9–1.9)	.18		
	5001–7500	**442**	**78 (18)**	**1.9 (1.3–2.9)**	**.001**		
	≤5000	**47**	**10 (21)**	**2.4 (1.1–5.2)**	**.02**		
Absolute lymphocyte count, cells/μL	>2000	624	74 (12)	1.0			
	1501–2000	533	63 (12)	1.0 (.7–1.4)	.98		
	1001–1500	349	52 (15)	1.3 (.9–1.9)	.18		
	≤1000	**144**	**33 (23)**	**2.2 (1.4–3.5)**	**.001**		
Absolute $CD4^+$ T cell count, cells/μL	>500	489	44 (9)	1.0			
	351–500	423	43 (10)	1.1 (.7–1.8)	.55		
	201–350	**446**	**65 (15)**	**1.7 (1.1–2.6)**	**.009**	**1.5 (1.1–2.2)**	**.02**
	≤200	**313**	**76 (24)**	**3.2 (2.2–4.9)**	**<.001**	**2.8 (2.0–4.0)**	**<.001**
Absolute $CD8^+$ T-cell count, cells/μL	>1500	179	21 (12)	1.0			
	1001–1500	489	58 (12)	1.0 (.6–1.7)	.96		
	501–1000	835	119 (14)	1.3 (.8–2.1)	.38		
	≤500	131	24 (18)	1.7 (.9–3.2)	.11		
HIV RNA load, $\log_{10}$ copies/μL	Undetectable	42	5 (12)	1.0			
	1.18–3.00	216	23 (11)	.9 (.3–2.5)	.81		
	3.01–4.00	552	75 (14)	1.2 (.4–3.1)	.76		
	4.01–5.00	659	85 (13)	1.1 (.4–2.9)	.85		
	>5.00	191	38 (20)	1.8 (.7–5.0)	.23		
Anti-syphilis antibody	No	1632	220 (13)	1.0			
	Yes	17	2 (12)	.9 (.2–3.8)	.84		
Anti-HCV antibody	No	1584	202 (13)	1.0			
	Yes	**75**	**23 (31)**	**3.0 (1.8–5.1)**	**<.001**	**2.6 (1.5–4.3)**	**.001**

Boldface text indicates significant values (≤.05).

Abbreviations: ALT, alanine aminotransferase; anti-HBc, antibody to hepatitis B core antigen; CI, confidence interval; HCV, hepatitis C virus; HIV-1, human immunodeficiency virus type 1; NS, not significant; OR, odds ratio; WBC, white blood cell.

[a] Logistic regression analysis was used.

[b] Multivariate logistic regression analysis was used.

women have been shown to have stable HBV DNA levels, with slight increases during late pregnancy [39], likely due to their relative immune-suppressed state. In our study, HBV DNA was measured in pregnant women during the last trimester. Our results are thus in favor of a low level of HBV replication in women with isolated anti-HBc. Second, infant HBV infection

Table 4. Factors Associated With Occult Hepatitis B Virus Infection in HIV-1–Infected Pregnant Women With Isolated Antibody to Hepatitis B Core Antigen

				Univariate Analysis		Multivariate Analysis	
Parameters	Category	No.	Occult HBV Infection (%)	OR (95% CI)	*P* Value[a]	OR (95% CI)	*P* Value[b]
Age at enrollment, y	≤25	83	20 (24)	1			
	26–30	57	12 (21)	.8 (.4–1.9)	.67		
	31–35	39	8 (21)	.8 (.3–2.1)	.66		
	>35	21	7 (33)	1.6 (.6–4.4)	.39		
Region of birth	Central	29	9 (31)	1			
	Eastern	27	4 (14)	.4 (.1–1.4)	.16		
	Northern	69	18 (26)	.8 (.3–2.0)	.62		
	Northeastern	62	16 (26)	.8 (.3–2.0)	.60		
	Southern	3	0 (0)	. . .	. . .		
	Western	10	0 (0)	. . .	. . .		
Previous pregnancy	No	75	16 (21)	1			
	Yes	124	31 (25)	1.2 (.6–2.4)	.56		
ALT, IU/L	≤30	165	42 (25)	1			
	31–60	22	2 (9)	.3 (.1–1.3)	.11		
	>60	6	2 (33)	1.5 (.3–8.3)	.67		
WBC count, cells/μL	>10 000	36	7 (19)	1			
	7501–10 000	80	17 (21)	1.1 (.4–3.0)	.82		
	5001–7500	70	20 (29)	1.7 (.6–4.4)	.31		
	≤5000	8	2 (25)	1.4 (.2–8.4)	.73		
Absolute lymphocyte count, cells/μL	>2000	62	13 (21)	1			
	1501–2000	55	16 (29)	1.5 (.7–3.6)	.31		
	1001–1500	45	13 (29)	1.5 (.6–3.7)	.35		
	≤1000	31	4 (13)	.6 (.2–1.9)	.35		
Absolute $CD4^+$ T-cell count, cells/μL	>500	40	10 (25)	1			
	351–500	37	8 (22)	.8 (.3–2.4)	.73		
	201–350	54	15 (28)	1.2 (.5–2.9)	.76		
	≤200	68	14 (21)	.8 (.3–2.0)	.60		
Absolute $CD8^+$ T-cell count, cells/μL	>1500	18	7 (39)	1			
	1001–1500	51	9 (18)	.3 (.1–1.0)	.07		
	501–1000	104	25 (24)	.5 (.2–1.4)	.19		
	≤500	22	5 (23)	.5 (.1–1.8)	.27		
HIV RNA load, $\log_{10}$ copies/μL	≤ 3.00	24	10 (42)	1			
	3.01–4.00	68	18 (26)	.5 (.2–1.3)	.17	.5 (.2–1.3)	.17
	4.01–5.00	71	15 (21)	.4 (.1–1.0)	.05	.4 (.1–1.0)	.05
	> 5.00	**34**	**4 (12)**	**.2 (.05–.7)**	**.013**	**.2 (.05–.7)**	**.013**
Anti-syphilis antibody	No	191	45 (24)	1			
	Yes	2	1 (50)	3.2 (.2–53)	.41		
Anti-HCV antibody	No	174	39 (22)	1			
	Yes	22	8 (36)	2.0 (.8–5.1)	.15		

Boldface text indicates significant values (≤.05).

Abbreviations: ALT, alanine aminotransferase; CI, confidence interval; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV-1, human immunodeficiency virus type 1; OR, odds ratio; WBC, white blood cell.

[a] Logistic regression analysis was used.

[b] Multivariate logistic regression analysis was used.

status was determined using HBV DNA PCR at 4 months of age. Because children in the original study (prevention of mother-to-child transmission of HIV-1) were only followed up until 12 months of age, it was not possible to reliably rule out a perinatal exposure to HBV based on anti-HBc testing. Indeed, anti-HBc immunoglobulin G, a marker of exposure to HBV, is

passively transmitted through placenta to the fetus and can persist in children several months before being completely cleared at 24 months of age [40].

In conclusion, our study shows that the prevalence of HIV-1–infected pregnant women presenting with isolated anti-HBc/occult HBV infection was low (2.6%) and that women with isolated anti-HBc and occult HBV infection have very low HBV DNA levels and are thus at very low risk to transmit HBV to their infants.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

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Retrovirology

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Oral presentation

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Transmission of Hepatitis B virus (HBV) minor variants in children born to HBV/HIV co-infected mothers

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Background

Since 1992, the Ministry of Public Health has integrated newborns HBV vaccination into the national expanded program on immunization. However, some children acquire HBV infection despite immunization.

Objective

To characterize HBV vaccine escape mutants in infants born to HBV/HIV-1 co-infected mothers.

Methods

Of 1433 HIV-infected women participating in the perinatal HIV prevention trial (PHPT-1), 107 were HBsAg positive. Five transmitted HBV to their children despite HBV vaccination in their children were documented. Blood samples collected from mothers during pregnancy and children at 4 and 6 months of age were analyzed by direct PCR sequencing of the S gene ("a" determinant region and flanking regions). HBV variants were sequenced after cloning of PCR products into pGEM-T® easy vector (20–25 clones by samples). Sequencing was performed using the BigDye Terminator V.3.1 sequencing kit, Applied Biosystem. Sequence alignments were performed using Bioedit software. HBV serotype was inferred from results at codons 122, 127, 160, 177 and 178 of the S gene.

Results

Complete samples series were available for 3 mother-child pairs, all infected by HBV genotype C. Infant virus direct sequencing showed no known vaccine escape mutation. However, direct sequencing identified the sK122R mutation in 2 infants but not in their mother. The predicted dominant HBV serotype in the 2 mothers was *adrq+*, while it was *ayr* in the 2 children at 4 months of age. Although sK122R was not detected by direct sequencing, further analysis of maternal clones showed that the 2 mothers harbored this minor variant at very low frequency (1 of 65 clones and 2 of 67 clones, respectively). Analysis of children HBV clones showed an increase of *ayr* variants from 4 months to 6 months.

Conclusion

Although the impact of the sK122R mutation on HBV vaccine escape is unknown, this study suggests that HBV minor maternal variants defining serotype can be transmitted to children who received HBV vaccine. This observation justifies the systematic virological evaluation of

children infected despite active immunization and their mother.

Abstract: O_18

Prevention of Mother-to-Child transmission

Prevalence of hepatitis B virus (HBV) infection in infants born to HIV/HBV co-infected women and factors associated with vertical transmission of HBV

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Introduction: Since the integration of Hepatitis B (HB) vaccine in the Expanded Program on Immunization (EPI) in Thailand in 1992, there has been no evaluation of hepatitis B virus (HBV) transmission in infants born to mothers co-infected with HIV-1 and HBV. We assessed the prevalence of HBV infection in infants born to HBV-HIV-1 co-infected women and determined risk factors associated with transmission of HBV.

Materials & Methods: HIV-1 infected pregnant women enrolled, between 1997 and 2010, in large nation-wide trials for perinatal HIV prevention were screened for HB surface antigen (HBsAg). HBsAg-positive women were then tested for HBeAg and HBV DNA quantification. Infants born to HBsAg-positive women were tested for HBsAg and/or HBV DNA within the first 6 months of age. Factors associated with vertical transmission of HBV were analyzed using Fisher's exact test for categorical variables and Mann–Whitney test for continuous variables. Factors included in analysis were maternal age at enrollment, alanine transaminase (ALT) level, CD4 T-cell count, HIV RNA load, HBV DNA load, HBeAg status, and infant gender.

Results: Of 3,747 HIV-infected women, 266 (7.1%) were HBsAg positive. Prior to delivery, median of maternal age was 26 years old (IQR: 22-29), ALT 17 IU/L (IQR: 12-26), CD4 T-cell count 355 cells/mL (IQR: 228-500), HIV RNA 4.02 $\log_{10}$ copies/mL (IQR: 3.36-4.59) and HBV DNA 4.76 $\log_{10}$ IU/mL (IQR: 1.84-7.70). Fifty-four percent of HBsAg positive mothers were HBeAg-positive. Thirteen of 251 evaluable children (5.2%; 95%CI 2.8-8.7) were infected with HBV. Ten infants were born to HBeAg-positive mothers, of whom, six were found to be infected at birth and were born to women with HBV DNA >7 $\log_{10}$ IU/mL. Three were born to HBeAg-negative women. None of the HBV infected infants were HIV infected. Women who transmitted HBV to their infants had higher median ALT level, as compared to non-transmitters (25 vs. 17 IU/L, p=0.02). High HBV DNA level and HBeAg positivity were associated with HBV transmission (p=0.056 and 0.096, respectively).

Conclusions: The prevalence of HBV infection in infants born to HBV-HIV-1 co-infected women was 5%. Maternal factors potentially associated with the risk of HBV vertical transmission were elevated ALT, HBV DNA levels, and HBeAg positivity. In addition to infant HB vaccination, other interventions targeting women with high HBV DNA or HBeAg positive are thus needed to further decrease transmission of HBV.

No conflict of interest

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- Full scholarship to attend the 4th Dominique Dormont International Conference, Maternal chronic viral infections transmitted to the infants: from mechanisms to prevention and care, Paris, France, December 13-15, 2007.
- A living allowance for technical training and conducting PhD research in France for one year from the Franco-Thai Cooperation Program in Higher Education and Research, November 2007 – October 2008.
- The scholarship for supporting doctoral thesis, Department of Capacity-Building for Scientific Communities of Developing Country, (Le bourse de soutien de thèse de doctorat (BSTD) du Département Soutien et Formation des communautés

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- International scholarship to attend the 18th conference on retroviruses and opportunistic infections (CROI), 27th February – 2nd March, 2011, Boston, Massachusetts, USA.
- Scholarship for supporting graduate student from Graduate School, Chiang Mai University, 2011

Publications

- Ngo-Giang-Huong N, **Khamduang W,** Leurent B, Collins I, Nantasen I, Leechanachai P, Sirirungsi W, Limtrakul A, Leusaree T, Comeau AM, Lallemant M, Jourdain G. Early HIV-1 Diagnosis Using In-House Real-Time PCR Amplification on Dried Blood Spots for Infants in Remote and Resource-Limited Settings. *Journal of Acquired Immune Deficiency Syndrome* 2008; 49 (5): 465-471.
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- **Khamduang W,** Sirirungsi W, Jourdain G, McIntosh K, Pagdi K, Somsamai R, Sirinontakan S, Hinjiranandana T, Ardong W, Lallemant M, Ngo-Giang-Huong N. Risk factors for Human Cytomegalovirus (HCMV) infection in infant born to HIV-1 infected mothers in Thailand. 4th International AIDS Society Conference

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Poster presentations

- Ngo-Giang-Huong N, Sirirungsi W, **Khumduang W,** Boonprasit W, Roederer T, Barin F, Lallemant M. HIV-1/HCV co-infection in pregnant women and their infants in Thailand. 2nd International AIDS Society Conference on HIV Pathogenesis and Treatment, Paris, France. July 13-16, 2003. (Abstract No. 994). *Antiviral Therapy* 2003; 8 (Suppl.1):S465
- Ngo-Giang-Huong N, Sirirungsi W, **Khamduang W,** Boonprasit W, Le Coeur S, Barin F, Lallemant M. Hepatitis C virus co-infection in HIV positive pregnant women and rate of HCV transmission to the infants in Thailand. 10th ASEAN Conference in Medical Laboratory Technology and the 28th Annual Meeting of the Association of Medical Technologists of Thailand, April 26-30, 2004, Chiang Mai, Thailand, (P22).
- Ngo-Giang-Huong N, Sirirungsi W, Boonprasit W, **Khamduang W,** Suwannachat B, Achalong J, Kovitanggoon K, Chotivanich N, Osalaraksa P, Layangool P, Thongdej R, Bhakeecheep S, Pipatnakulchai S, Barin F, Lallemant M J. Transmission of Hepatitis B Virus from HIV Co-infected Mothers to their Infants in Thailand, 15th International AIDS Conference, 11-16th July 2004, Bangkok, Thailand. Abstract no. MoPeB3331.
- **Khamduang W,** Kanthawong S, Leechanachai P, Ananpatharachai P, Attavijrakarn P, Wannarit P, Hanpinitsak S, Hotrawarikarn S, Sirinontakan S, Sriojana S, Tanupattarachai S, Hinjiranandana T, Ardong W, Ngo-Giang-Huong N. Early Detection of HIV infection in children born to HIV positive mothers by using real time PCR, 15th International AIDS Conference, 11-16th July 2004, Bangkok, Thailand. Abstract no. MoPeB3162.

- **Khamduang W,** Gaudy-Graffin C, Moreau A, Ngo-Giang-Huong N, Jourdain G, Lallemant M, Pipatnakulchai S, Putiyanun C, Kunkongkapan S, Wannarit P, Sirinontakan S, Ardong W, Sirirungsi W, Goudeau A. Transmission of Hepatitis B virus (HBV) minor variants in children born to HBV/HIV co-infected mothers. 12th National AIDS Conference, 27-29th May, 2009, Bangkok, Thailand. (CP08)
- **Khamduang W,** Gaudy-Graffin C, Moreau A, Ngo-Giang-Huong N, Jourdain G, Lallemant M, Pipatnakulchai S, Putiyanun C, Kunkongkapan S, Wannarit P, Sirinontakan S, Ardong W, Sirirungsi W, Goudeau A. Hepatitis B virus (HBV) virological response to combination antiretroviral treatment includes lamivudine (3TC) in HIV/HBV co-infected individuals in Thailand. International Meeting; The molecular biology of hepatitis B viruses, 30th August - 2nd September, 2009, Tours, France. (P-20)
- **Khamduang W,** Gaudy-Graffin C, Ngo-Giang-Huong N, Jourdain G, Lallemant M, Pipatnakulchai S, Putiyanun C, Kunkongkapan S, Wannarit P, Sirinontakan S, Ardong W, Sirirungsi W, Goudeau A. The low prevalence of occult Hepatitis B infection in HIV-1 infected pregnant women with antibody to hepatitis B core antigen alone in Thailand. 18th international AIDS conference, 18-23th July, 2010, Vienna, Austria. (THPE0205)
- **Khamduang W,** Gaudy-Graffin C, Ngo-Giang-Huong N, Jourdain G, Moreau A, Luekamlung N, Halue G, Buranawanitchakorn Y, Kunkongkapan S, Buranabanjasatean S, Sureau C, Lallemant M, Sirirungsi W, Goudeau A and the Program for HIV Prevention and Treatment (PHPT) group. Hepatitis B Escape Mutants in Infants Born to Human Immunodeficiency Virus (HIV)-infected Mothers Co-infected with Hepatitis B Virus (HBV). The 21st Conference of the

Asian Pacific Association for the study of the liver (APASL), 17-20th February, 2011, Bangkok, Thailand. (PP06.41)

- **Khamduang W,** Gaudy-Graffin C, Ngo-Giang-Huong N, Jourdain G, Moreau A, Luekamlung N, Halue G, Buranawanitchakorn Y, Kunkongkapan S, Buranabanjasatean S, Lallemant M, Sirirungsi W, Goudeau A, and the Program for HIV Prevention and Treatment (PHPT) group. Long-term virological response of Hepatitis B virus to lamivudine-containing HAART in patients co-infected with HIV and HBV in Thailand. The 13th Thai national AIDS seminar, 29th – 31st March, 2011, Bangkok, Thailand. (AP3)
- **Khamduang W,** Gaudy-Graffin C, Ngo-Giang-Huong N, Jourdain G, Moreau A, Luekamlung N, Halue G, Buranawanitchakorn Y, Kunkongkapan S, Buranabanjasatean S, Lallemant M, Sirirungsi W, Goudeau A, for the Program for HIV Prevention and Treatment (PHPT) study group. Long-term virological response of Hepatitis B virus (HBV) to lamivudine-containing HAART in patients co-infected with HIV and HBV in Thailand. The XIX international AIDS conference, 22-27th July 2012, Washington DC, USA. (WEPE049)

Invited speaker

- **Khamduang W.** Franco-Thai Highlight: Hepatitis B Vaccine Escape. The International Workshop on "Interdisciplinary Approach to the Management of HIV: A Model for other Infectious Diseases", 16th – 18th March, 2011, Chiang Mai, Thailand.
- **Khamduang W.** The interrelated transmission of HIV-1 and cytomegalovirus during gestation and delivery in the offspring of HIV-infected mothers. The

International Workshop on “Interdisciplinary Approach to the Management of HIV: A Model for other Infectious Diseases”, 16th – 18th March, 2011, Chiang Mai, Thailand.

- **Khamduang W.** Situation of HBV infection in Thailand. The Thai National Community Advisory Board meeting, 9th February 2012 Chiang Mai, Thailand.
- **Khamduang W.** Evaluation of HBV residual transmission to infants in South-East Asia. The Laotian National Workshop on Viral Hepatitis, 29th February, 2012, Vientiane, Laos.
- **Khamduang W.** Residual perinatal transmission of HBV in the context of HBV immunoprophylaxis. A Research Agenda for Diagnosis, Prevention and Treatment of HIV, HBV, and HPV Infections in Southeast Asia. 14th – 15th March 2012, Chiang Mai, Thailand.
- **Khamduang W.** HIV-HBV co-infections in adults: results and research questions. The PHPT co-investigators, nurses and counselors meeting. 28th – 29th August 2012, Bangkok, Thailand.